The Association of Interoceptive Awareness with Alexithymia and Empathy:

A Multimethodal Investigation of Neural Activity Patterns and Neurotransmitter Concentrations in Anterior Cingulate Cortex and Insula

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Table of contents

Abbreviations

ACC Anterior Cingulate Cortex

ANR Awareness of Autonomous Nervous System Reactivity

BOLD Blood Qxygen Level Dependent

BPQ Body Perception Questionnaire

Cr Creatine

CSF Cerebrospinal Fluid

DDF Difficulty Describing Feelings

DIF Difficulty Identifying Feelings

DMPFC Dorsomedial Prefrontal Cortex

EaE Empathy after Exteroception

EaI Empathy after Interoception

EPI Epi Planar Imaging

EOT Externally Oriented Thinking

Extero Exteroception

fMRI Functional Magnetic Resonance Imaging

GABA Gamma-aminobutyric Acid

Gln Glutamine

Glu Glutamate

Hb Haemoglobin

IA Interoceptive Awareness

Intero Interoception

MPC Medial Parietal Cortex

MRS Magnetic Resonance Spectroscopy

NAA N-acetylaspartic Acid

PCC Posterior Cingulate Cortex

PCr Phosphocreatine

RF Radio Frequency

SACC Supragenual Anterior Cingulate Cortex

TAS Toronto Alexithymia Scale

TE Echo Time

List of figures

1 Background and aims

2 Empirical part

Study 1

Study 2

List of tables

1 Background and aims

2 Empirical part

Study 2

Summary

Interoceptive awareness (IA) is the awareness of bodily signals and has been highlighted as important in many early theories of emotion. William James was one of the first to present a psychological theory linking viscero-afferent feedback to emotional experience. The processing of bodily and thus interoceptive stimuli may be a crucial component in yielding empathy, since affective states are often assumed to involve awareness of one´s own bodiliy state. The assumption of a close relationship between interceptive awareness and empathy is further supported when considering the regions recruited during both processes, like the insula and the anterior cingulate cortex. Although especially the affective component may implicate interoception and interoceptive awareness, the impact of interoception on empathy has never been evaluated behaviorally or neurophysiologically.

Also associated with altered insula and anterior cingulate cortex (ACC) function is Alexithymia. Alexithymia, a personality trait, is marked by cognitive and affective features including difficulties in identifying and describing feelings as well as in distinguishing feelings from bodily sensations of emotional arousal. Recent studies found a negative correlation between prefrontal glutamate (Glu) and mental perspective taking as well as extraversion. Even though several functional imaging studies investigated the neuronal signatures of interoceptive awareness and the personality trait alexithymia, little is known about the functional relevance of neurotransmitter concentrations.

Despite the (1) specific role of interoceptive awareness in alexithymia and empathic processing, the (2) functional relevance of ACC and insula for interoceptive awareness and empathy, (3) the association of alexithymia with structural and functional changes in ACC and insula and (4) the behavioral relevance of altered neurotransmission in these regions, neither the relationship between interoceptive awareness, empathy and alexithymia nor their respective association with neuronal acitivity and neurotransmitter concentrations in ACC and insula has been elucidated yet.

The first study demonstrates for the first time a direct interaction between interoception and empathy. Our data show that preceding interoceptive awareness enhances neural activity in bilateral insula and various midline regions during empathy. The enhancement of neural activity during empathy in both interoceptive and empathy networks by preceding interoceptive awareness suggests a close relationship between interoception and empathy; thereby, interoception seems to be implicated to empathy.

In the second study we examined the relationship between alexithymic facets as assessed with the Toronto Alexithymia Scale (TAS-20) and interoceptive awareness (assessed with the Body Perception Questionnaire) and investigated their association with glutamate and gamma-aminobutyric acid (GABA) concentrations in the left insula and the ACC using 3-Tesla proton magnetic resonance spectroscopy. Behaviorally, we found a close association between alexithymia and interoceptive awareness. Furthermore, glutamate levels in the left insula were positively associated with both alexithymia and awareness of autonomic nervous system reactivity, while GABA concentrations in ACC were selectively associated with alexithymia.

Zusammenfassung

Interozeptives Bewusstsein ist das Bewusstsein von Körpersignalen und spielte schon in vielen frühen Theorien der Emotion eine wichtige Rolle. William James war der Ansicht, dass Gefühle die Folge viszeraler Veränderungen sind, die bei der Wahrnehmung von emotionsauslösenden Sachverhalten auftreten. Die Verarbeitung der körperlichen und damit interozeptiven Reize könnte auch eine entscheidende Komponente der Empathie sein. Die Annahme einer engen Beziehung zwischen interozeptivem Bewusstsein und Empathie wird durch die Betrachtung gestützt, dass bei beiden Prozessen gleiche Hirnregionen involviert sind, wie zum Beispiel die Insula und der anteriore cinguläre Kortex. Obwohl vor allem die affektive Komponente bei Interozeption und interozeptiven Bewusstsein von grosser Bedeutung ist, wurde der Einfluss von Interozeption auf Empathie nie behavioral oder neurophysiologisch untersucht.

Veränderte Insula und anteriore cinguläre Cortex (ACC)-Funktion ist auch bei Alexithymie zu finden. Alexithymie ist ein Persönlichkeitsmerkmal, das durch Probleme in kognitiven und affektiven Funktionen, Probleme bei der Identifizierung und Beschreibung von Gefühlen sowie bei der Unterscheidung von körperlichen Empfindungen oder emotionaler Erregung gekennzeichnet ist. Aktuelle Studien fanden eine negative Korrelation zwischen präfrontalen Glutamat (Glu) und Perspektivenübernahme sowie Extraversion. Auch wenn mehrere funktionelle Bildgebungsstudien die neuronalen Änderungen bei interozeptivem Bewusstsein und Alexithymie untersucht haben, so ist doch wenig über die Bedeutung der Neurotransmitterkonzentrationen bekannt.

Trotz der (1) besonderen Rolle von interozeptivem Bewusstsein bei Alexithymie und Empathie, der (2) funktionellen Relevanz von ACC und Insula bei interozeptivem Bewusstsein und Empathie, (3) der Assoziation von Alexithymie mit strukturellen und funktionellen Veränderungen im ACC und Insula und (4) der Relevanz von veränderter Neurotransmission in diesen Regionen wurde bisher weder die Beziehung zwischen

interozeptivem Bewusstsein, Empathie und Alexithymie noch ihre jeweilige Assoziation mit neuronaler Aktivität und Neurotransmitterkonzentrationen im ACC und Insula untersucht.

In der ersten Studie zeigen wir zum ersten Mal eine direkte Wechselwirkung zwischen Interozeption und Empathie. Die Ergebnisse unserer fMRI- Untersuchung zeigen, dass bei Interozeption die neuronale Aktivität in der bilateralen Insula und verschiedenen medialen kortikalen Regionen während Empathie verstärkt ist.

In der zweiten Studie untersuchten wir den Zusammenhang zwischen Facetten der Alexithymie, die durch die Toronto Alexithymie - Skala (TAS- 20) aufgezeigt wurden, interozeptivem Bewusstsein (ermittelt mit dem Körperwahrnehmungs-Fragebogen, BPQ) und den Konzentrationen von Glutamat und Gamma-Aminobuttersäure (GABA) -Konzentrationen in der linken Insula und dem ACC, die mithilfe der 3- Tesla- Magnetresonanzspektroskopie bestimmt wurden.

Auf der Verhaltensebene fanden wir eine enge Verbindung zwischen Alexithymie und interozeptivem Bewusstsein. Weiterhin war die Glutamatkonzentration in der linken Insula positiv sowohl mit Alexithymie als auch mit dem Subscore „Reaktionsfähigkeit des Autonomen Nervensystems“ des BPQ assoziiert, während GABA -Konzentrationen im ACC selektiv mit Alexithymie verbunden waren.

1 Background and aims

1.1 The role of interoceptive awareness in empathy and alexithymia

Interoception refers to sensitivity to stimuli originating from within the body and is a term, which has been first introduced by Sherrington. He originally distinguished Interoception from the sensing of stimulation from outside the body (exteroception) and of the body's position in space (proprioception) (Sherrington, 1948). In nowadays, the term is used to include the physiological condition of the entire body and the ability of visceral afferent information to reach awareness and affect behaviour, either directly or indirectly. The system of interoception as a whole constitutes "the material me", and relates to how we perceive feelings from our bodies that determine our mood, sense of well-being and emotions. This ability, to consciously detect subtle changes in bodily states including temperature, pain, visceral or muscular sensations, is referred to as "interoceptive awareness" (IA), which varies between individuals. IA is mostly measured using the heatbeat task, aiming at the individuals's ability to accurately perceive their own heartbeat (Critchley et al., 2004). William James (1884) was among the first to postulate, that the feelings from our bodies are the basis for self-awareness and emotion, stating that "bodily changes follow directly the perception of the exciting fact, and that our feelings of the same changes as they occur is emotion…. that we feel sorry because we cry, angry because we strike, afraid because we tremble…" (James, 1890). In short, he hypothesized that the feedback from the body produced the feeling part but not necessarily the perceptual-cognitive or behavioral parts. Based on this theory, Damasio formulated the somatic marker hypothesis in which he refers to the obligatory body relatedness of feelings, stating "the body is the main stage for emotions, either directly or via its representations in somatosensory structures of the brain (Damasio, 1999).

Besides the somatosensory cortex, also the cingulate cortex, frontal cortex and insular cortex

are implicated in interoception. Especially the anterior insular cortex has been shown to be of relevance for interoception (Critchley et al., 2004; Pollatos et al., 2007a) and the re-representation and integration of interoceptive bodily signals with higher order and emotional processes (Craig, 2003, 2009). The anterior insula has also been the focus of a study by Zaki et al. (2012) where he showed that neural activity during emotional processing and interoceptive awareness overlaps in the very same region, showing a convergence zone for the representations of the body and emotion. In affective disorders, studies have reported alterations in interoceptive awareness and insula activity (Wiebking et al., 2011; Dunn et al., 2007). As mentioned above, interoceptive awareness has been mostly quantified using heartbeat perception tasks that measure the ability to perceive one's heartbeat (Critchley et al., 2004; Herbert et al., 2007; Pollatos et al., 2007b; Wiens, 2005, Wiebking et al., 2011). It was shown that neural activity during interoceptive awareness was increased in brain regions including the anterior cingulate cortex and insular cortex. Further studies demonstrated that IA is positively related to (a) more intense processing of emotionally arousing stimuli (Herbert et al., 2007; Pollatos et al., 2005b) and (b) greater activation of the insular cortex, the anterior cingulate cortex (ACC), the ventromedial/dorsolateral prefrontal cortex, and the somatosensory cortices (Critchley et al., 2004; Pollatos et al., 2007). These brain structures are relevant for monitoring the internal emotional and viscerosensory state (Critchley et al., 2000; Critchley et al., 2003; Thayer and Broschot, 2005), for emotion processing and emotional reactivity (Craig, 2002; Gray et al., 2007; Phan et al., 2002; Thayer and Broschot, 2005), as well as for the feeling of self-generated and externally induced emotions (Anders et al., 2004; Damasio et al., 2000). Furthermore, influential interpretations of central neuroanatomical pathways back up the role of the insula, in conjunction with anterior cingulate cortex, in the representation and control of internal states (Craig, 2002).

The brain structures mentioned above that have been demonstrated to be of importance for the processing of interoceptive signals are the same brain structures that show altered

activation and morphology in alexithymia (Borsci et al., 2009). Alexiythmia literally means "no words for emotion," and comes from the Greek *a* for "lack" *lexis* for "word" and *thymia* for "emotion." Its' concept was introduced by Sifneos (1973) after observing patients with classic psychosomatic diseases who failed to respond to dynamic psychotherapy. This multi-facet personality trait is characterized by marked deficits in the ability to identify and describe one's feelings (Lumley et al., 2007). It is associated with poor quality of life (Mattila et al., 2009) and with somatization, the conversion of psychological distress into physical symptoms (Mattila et al., 2009) as well as increased individual and interpersonal distress (Humphreys et al., 2009). Its prevalence rate varies from 13% (Salminen et al., 1999) to 19% (Todarello et al., 1995). As defined by Nemiah, Freyberger and Sifneos (1976), the salient features of alexithymia are difficulties in identifying and describing feelings and in distinguishing between feelings and bodily sensations of emotional arousal. Since then it has been characterized by emptiness of feelings (Sifneos, 1991), poverty of imagination or of a fantasy life (Haviland and Reise, 1996), difficulties in communicating with other people (Sifneos, 1991), lack of positive emotions and high prevalence of negative emotions (Taylor, 1984). Difficulties distinguishing the bodily changes associated with emotional reactions make it hard to use the signalling value of emotions – termed 'somatic markers' by Damasio (1995) – in everyday life to identify patterns and inform decision-making. To measure alexithymia the most-widely used instrument is the twenty-item Toronto Alexithymia Scale (TAS-20) (Bagby, Parker, & Taylor, 1994). It has three subscales: 1) Difficulty identifying feelings and distinguishing them from bodily sensations (DIF), 2) difficulty expressing feelings (DEF), and 3) externally-oriented thinking (EOT). On a structural and neural level, as mentioned above, the ACC and insula are brain regions that are relevant in generating emotional experience and alexithymia-related difficulties in perceiving and experiencing emotions may be associated with dysfunction of the ACC, insula, amygdala and striatum (van der Velde et al., 2013). Furthermore, subcortical areas, such as the amygdala and striatum, are proposed to underlie emotion-processing difficulties in alexithymia because of their role in the detection of emotional significance and

the generation of emotional feelings (Kano and Fukudo, 2013, Moriguchi and Komaki, 2013). Alexithymia is considered to be a risk factor for various psychiatric and psychosomatic disorders, including depression (Speranza et al., 2005; Lipsanen et al., 2004). Several studies suggested that alexithymia represents a stable risk factor for depression and that both, alexithymia and depression, are different constructs (Honkalampi et al., 2000, Luminet et al., 2007). However, mechanisms underlying the association between alexithymia and depression are not clear yet.

Moreover, given the multi-faceted nature of alexithymia, differential effects of its dimensions have been generally highlighted: in some studies, an association with depression was found only for DIF and DDF, but not for EOT (Haviland et al., 1988, Hendryx et al., 1991). In other studies, only an association between DIF and depression or negative affect has been found (Bailey and Henry, 2007, De Berardis et al., 2008), whereas the association between DDF and depression turned out to be much more unstable (Bailey & Henry, 2007). These heterogeneous findings are not surprising if one takes into account that alexithymia differs from the cognitive distortions of depression as measured by the BDI (Komaki, 2013). Recent studies have also found severe degrees of alexithymia in about 50% of individuals with autism spectrum conditions (Silani et al., 2008). This study furthermore showed that the degress to which participants were able to understand their own emotions (i.e. their degree of alexithymia) was correlated with activity in the anterior insula during an interoceptive task. Scores on a self-report measure of trait empathy were also correlated with the participants' self- reported degree of alexithymia and activity in the anterior insula, when introspecting on emotion.

The shared network models of empathy also predict the association between alexithymia and empathy by suggesting that the networks responsible of processing emotions in the self are the same networks used to represent the emotions of others.

Many people with alexithymia also have deficits in emotional empathy (Bernhardt and Singer,

2012; Moriguchi et al., 2007). Empathy is a multidimensional construct (Davis, 1994) and refers to a constellation of related abilities: the tendency to take on or share the feelings of others, the ability to cognitively understand those feelings and the tendency to act pro-socially on the basis of those feelings (Decety and Batson, 2007; Zaki and Ochsner, 2009). Deficits in insight into one's own emotional state may constrain the capacity for understanding emotions in others. Responses to stimulation (pain), including autonomic reactions, have been proposed to be important for empathic awareness (Preston and de Waal, 2002). Activity within the anterior cingulate and the insular cortices increases during empathic responses (Bernhardt and Singer, 2012; Guo et al., 2012; Han et al., 2009; Lamm et al., 2011; Singer et al., 2004), and both regions contribute to the control of autonomic arousal (Critchley, 2009). Individual differences in emotional empathy are related to differences in the reactivity of the anterior cingulate and insular cortices (Krach et al., 2011; Masten et al., 2011; Pfeifer et al., 2008) in a manner similar to alterations in the reactivity reported in some studies of alexithymia (Berthoz et al., 2002; Heinzel et al., 2010).

As described above, even though several functional imaging studies investigated the neuronal signatures of interoceptive awareness and the personality trait alexithymia, little is known about the functional relevance of neurotransmitter concentrations. Previous studies reported that healthy subjects with the personality traits extraversion and harm avoidance showed decreased and increased anterior cingulate cortex (ACC) γ- aminobutyric acid (GABA) concentrations (Goto et al., 2010; Kim et al., 2009). Of specific relevance with regard to the investigation of alexithymia are findings of a negative correlation between prefrontal glutamate (Glu) and mental perspective taking as well as extraversion (Montag et al., 2008; Grimm et al., 2012), since alexithymia is marked by difficulties in identifying feelings as well as by limited experience of positive emotions (Sifneos, 1987). Further studies have reported abnormal interoceptive awareness, altered insula activity as well as modulation of functional connectivity between insula and ACC by glutamatergic neurotransmission in ACC (Horn et al.,

2010, Wiebking et al., 2011, Grimm et al., 2012) in affective disorders. Increased glutamatergic neurotransmission in the insula has been reported in acute and chronic pain (Gutzeit et al., 2011, Gussew et al., 2010, Harris et al., 2008) and been discussed as an indicator for amplified interoceptive sensory processing (Gutzeit et al., 2011; Gussew et al., 2010; Harris et al., 2008), High interoceptive awareness, which in turn might be related to a Glu- mediated increase in insula activity, has been associated with higher emotional arousal (Wiens et al., 2001; Pollatos 2005). Alexithymics show increased vulnerability to affective disorders (Leweke et al., 2012; Luminet, 2010), which are characterized by dysfunctional glutamatergic and GABA- ergic neurotransmission (Sanacora et al., 2008; Mathew et al., 2008; Walter et al., 2009; Hashimoto et al., 2010; Grimm et al., 2012).

Despite the specific role of both insula and ACC in alexithymia as well as in interoceptive awareness the respective relationships have not been elucidated yet. Therefore, we first aimed to investigate the association of interoceptive awareness with alexithymic features. Given the behavioral relevance of altered neurotransmission in Insula and ACC and their known altered activation in alexithymia and interoceptive awareness we applied spectroscopy to examine the association between alexithymic features, interoceptive awareness and glutamate and GABA concentrations in the left insula and ACC of healthy subjects.

Taken together, despite the (1) specific role of interoceptive awareness in alexithymia and empathic processing, the (2) functional relevance of ACC and insula for interoceptive awareness and empathy, (3) the association of alexithymia with structural and functional changes in ACC and insula and (4) the behavioral relevance of altered neurotransmission in these regions, neither the relationship between interoceptive awareness, empathy and alexithymia nor their respective association with neuronal acitivity and neurotransmitter concentrations in ACC and insula has been elucidated yet.

1.2 Methodological introduction

Functional magnetic resonance imaging (fMRI) is the method of choice, if one wants to investigate the relation between cognitive processes and the activation of underlying brain areas. During the past 23 years, since the first study was published in 1991, this method has become very popular, mostly based on the advantages compared to other imaging methods and for the reason of being non-invasive. Furthermore, this method allows an unexcelled spatial resolution of about 2 to 3 mm and a temporal resolution of a few seconds. Going a step further, to investigate concentrations of key chemicals in the brain, researchers are using magnetic resonance spectroscopy (MRS). This technique reveals concentrations of the chemicals througout the brain or localized areas in the brain, like used in study 2.

1.2.1 Functional magnetic resonance imaging (fMRI)

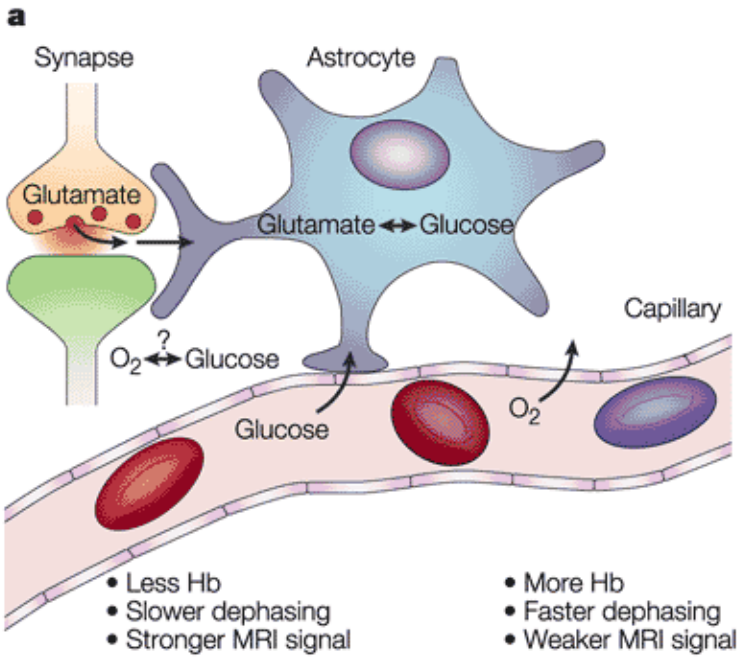
Functional magnetic resonance imaging (fMRI) is an imaging method developed to demonstrate regional, time-varying changes in brain metabolism and is currently the mainstay of neuroimaging in cognitive neuroscience. Before I go into detail about the physiology of fMRI, I will give a short summary of the basics of MRI.

The magnetic filed in an MRI scanner is generated by surrounding a coil of wire with super cooling fluids (liquid helium and liquid nitrogen) lowering the temperature to about 10°K. An extremely large magnetic field is created by an electrical current moving very fast in the coil. Magnetic resonance imaging (MRI) takes advantage of the high prevalence ofhydrogen in the body and the magnetic properties of the proton in a hydrogen atom. Hydrogen atoms induce a small magnetic field due to the spin of this atoms' proton. If one places the subject in a scanner, the subject's protons will align with the direction of the magnetic field and the subject becomes polarized. Under normal conditions, nuclear magnetic dipoles in the body are randomly distributed, which results in zero magnetization. A radio frequency (RF) pulse is then used to tip the protons out of alignment with the scanner's magnetic field. Protons have

„spins“, which have an orientation and a frequency. When the appropriate RF pulse frequency is chosen (Larmor frequency), the spins absorb energy and change orientation. Once the RF pulse is turned off, protons return to their original orientations and emit energy in form of radio waves. The emitted radio waves are measured. The T1 time constant describes how quickly the protons realign with the magnetic field (longitudinal magnetization, spin-lattice relaxation) and T2 time constant describes how quickly the protons emit energy when recovering to equilibrium (transverse magnetization, spin-spin relaxation). In T1 weighted images, fat in tissue has a high signal and is therefore represented by bright grey-scales, whereas cerebrospinal fluid (CSF), which contains little fat, has a low signal and is therefore represented in dark scales. The opposite is the case in T2 weighted images, where CSF is bright and fat dark. Two factors contribute to the decay of transverse magnetization in T2, namely the molecular interactions and the local inhomogeneities of the magnetic field. The combined time constant is called T2*. Echo Planar Imaging (EPI) technique is sensitive to changes in T2* and from it's measurements a spatial image can be constructed (Weishaupt et al., 2009).

Since the main interest of cognitive neuroscientists lies not so much in the anatomical structures of the brain, but in its functional behavior (i.e. its "activity"), T2* weighted images can be used to obtain functional measurements of brain activity. The key mechanism for functional images is the Blood Oxygenation Level Dependent (BOLD) contrast. The BOLD (Blood Oxygenation Level Dependent) mechanism is based on a signal change that is due to the hemodynamic and metabolic sequelae of neuronal responses. Hemoglobin without bound oxygen molecules, deoxyhemoglobin, is paramagnetic because of the high spin state (S = 2) of the heme iron. In contrast, oxygen-bound hemoglobin, oxyhemoglobin, has low spin (S = 0) and is diamagnetic (Pauling and Coryell, 1936) and is magnetically indistinguishable from brain tissue. However, the presence of deoxyhemoglobin in red blood cells makes their magnetic susceptibility different from the diamagnetic plasma in blood and, similarly, induces a difference in magnetic susceptibility between the blood and the surrounding tissue. In the

large homogenous magnetic fields used in MRI, compartmentalized susceptibility differences induce small magnetic field distortions in the blood as well as in the surrounding extra-vascular area. The BOLD signal displays a characteristic temporal profile. After the presentation of a stimulus, in some cases an initial decrease in the fMRI signal, known as the "initial dip", can be observed (Buxton et al., 2004). The BOLD-signal then rises and peaks 4-6 seconds after stimulus and returns to baseline after approximately up to 30 seconds. But the exact causal link leading from neuronal activity to changes in oxygen concentration in the blood and changes in regional cerebral blood flow (rCBF) is not totally understood yet. The current view is that the neuronal processes causing BOLD signal changes are associated with synaptic inputs at the site of activation, not with the output level of firing of the neuron receiving synaptic inputs (Logothetis et al., 2001). This means that fMRI reflects the synaptic activity driving neuronal assemblies, but cannot disclose the information content of the neuronal firing patterns produced by the neurons. The coupling to synaptic activity is very tight, so it is obvious that the metabolic load change is coupled directly to the neuronal activation. Furthermore, the vascular response is also coupled very tightly. One of those coupling mechanisms involves glutamate released for synaptic activation, which induces a change in Ca^{2+} in neighboring astrocytes. This result in the release of blood vessel dilators (at the contact point of astrocyte to arterioles and through rapidly diffusible substances like Nitric Oxide) and an increase in cerebral blood flow (CBF). Such tight coupling of BOLD signal to synaptic activity allows us to use the fMRI signal to probe functional responses in the brain, even though the response time of the BOLD signal (or any other signals based on vascular changes) takes several seconds and is much slower than the underlying neuronal processes (see **Box 1**).



Box 1 Haemodynamic Response

The figure shows the proposed relationship between synaptic activity, neurotransmitter recycling and metabolic demand (Heeger and Ress, 2002).

1.2.2 Magnetic Resonance Spectroscopy (MRS)

MRS provides a non-invasive diagnostic tool for the biochemical characterization of physiological processes in the brain. It is an analytical technique that can be used to complement the described above Magnetic Resonance Imaging (MRI) in the characterization of tissue. Both techniques use signals from hydrogen protons (1H), but MRI uses the information to create 2-dimensional images of the brain, while MRS uses 1H signals to determine the relative concentrations of target brain metabolites. Furthermore, MRS needs a step that ensures the homogeneity of the magnetic field, which is accomplished by “shimming”. Shimming is due to the fact, that magnetic fields obey the superposition principle and, therefore, the effective magnetic field present during the scan can be manipulated

through addition of correction fields. An ideal shim field exactly resembles the field variationto be compensated for in shape and amplitude, however, at reversed polarity. The application of such shim field fully removes the field imperfection and results in a homogeneous magnetic field distribution, i.e. constant field amplitude throughout the considered region-of-interst. Spectral overlap and disciminability of metabolites strongly depend on shim quality. Water is the most dominant molecule in the brain, so its concentration exceeds the concentrations of molecules of interest by a factor of 10000 or more. Therefore, suppression of the water signal is very essential and is achieved by the addition of water suppression pulses. MRS studies can be carried out for a single voxel or for a matrix ov voxels. **Study 2** used the single voxel approach, with voxel referring here to the volume of tissue being investigated. Single voxel spectroscopy allows evaluation of only small volumes of tissue and acquisition of quantitative data. Concentrations are usually expressed in arbitrary units or as ratios. The proton MR spectrum comprises a set of resonances (peaks) distributed along the x-axis, labelled in parts per million (ppm). The amplitude of the resonances is measured on the y-axis typically using an arbitrary scale. Although the positions of the resonances along the x-axis are constant, the relative heights of the resonances can differ depending on various MR imaging parameters.

As an internal standard in MR spectroscopy from which metabolite ratios can be calculated, most studies use creatine, as it is assumed to be relatively constant across the brain with little variation. The primary creatine resonance is located at ~3.02 ppm and is a combination of at least two compounds: creatine (Cr) and phosphocreatine (PCr) (Rosen et al., 2007). As both chemicals are related to ATP reserves, the peak is believed to be a marker of energetic metabolism (Miller et el., 1999). Creatine can be detected when long echo times (135 to 280ms) are being used. The parameter echo time (TE) controls the delay from the radiofrequency excitation pulse to the detection of the echo.

Since this thesis focuses on GABA (γ-aminobutyric acid) and glutamate, only these metabolites are described below.

Glutamate

Glutamate (Glu) along with glutamine (Gln) appears as multiple resonances between ~2.12 and ~2.35 ppm (β-γ region) as well as ~3.74 and ~3.75 ppm (α-region) (Mountford et al., 2010).

Glutamate is the most abundant neurotransmitter in the brain. In astrocytes, glutamate is oxidatively degraded or converted to glutamine by the astrocyte-specific enzyme glutamine synthetase. Smaller quantities of glutamine are also synthesized de novo or from GABA (Bak et al., 2006). Glutamine is released from astrocytes, accumulated by neurons and converted to glutamate by the neuron-specific enzyme phosphate-activated glutaminase and it is the major precursor for neuronal glutamate and GABA (Hertz and Zielke, 2004). The molecular structures of Glu and Gln are very similar and, as a result, give rise to similar magnetic resonance spectra. Thus, even though Glu has a relatively high concentration in the brain, its spectral features are usually contaminated by contributions from Gln, GABA, glutathione (GSH) and N-acetylaspartate (NAA).

GABA (γ-aminobutyric acid)

GABA is the main inhibitory neurotransmitter in the brain and is normally synthesized from glutamate in GABAergic neurons. GABA und glutamate keep the electrical balance in the human brain. GABA is present in the human brain at a concentration of about 1 mM, which is an order of magnitude lower than some of the more concentrated metabolites and ~40,000 times lower than water. GABAs three different multiplets correspond to the three methylene (CH2) groups in the molecule. These signals are overlapped by more intense signals arising from the more abundant metabolites NAA at 2 ppm, Cr at 3 ppm and glutamate (Glu) and glutamine (Gln) at 2.3 ppm (Total Glu + Gln is often referred to as Glx) (see **Figure 1**). GABA is found in two major pools within neurons and is thought to have a number of roles within the brain. Cytoplasmic GABA, primarily produced from glutamate via the tonically active 67 kD form of glutamic acid decarboxylase (GAD), is found throughout the neuron and is therefore hypothesised to have a role in metabolism (Martin et al., 1993). Vesicular GABA is found in

high concentrations within the pre-synaptic boutons; its concentration is controlled in the main via the active 65 kD GAD and it plays a role in inhibitory synaptic neurotransmission (Martin et al., 1993).

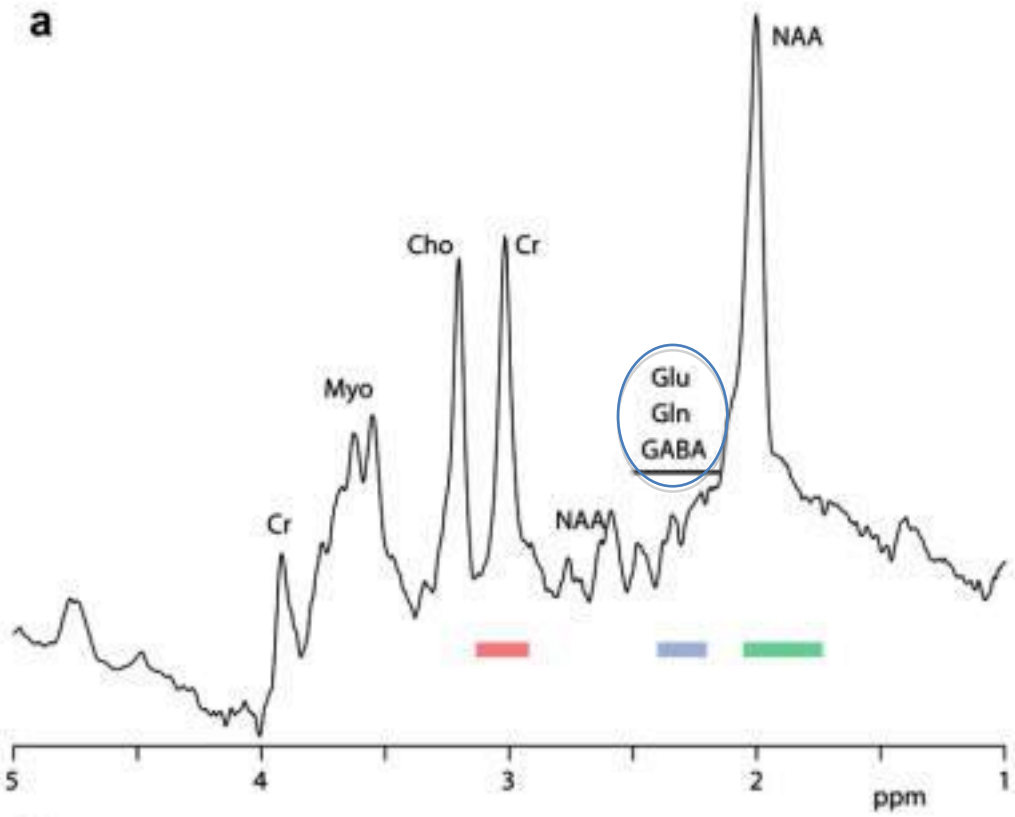


Figure 1: Chemical shift

Normal spectral data obtained at short-echo time by single-voxel 1H magnetic resonance (Puts and Edden, 2012). Blue circle marks the three metabolites γ-aminobutyric acid (GABA), glutamate (Glu) and glutamine (Gln) that are mentioned in the following studies.

1.3 Aims of the current thesis

In the current thesis, the first aim was to investigate how empathy-related neural activity in the interoceptive network is modulated by preceding interoceptive awareness and whether regions of the empathy network are differentially modulated during empathy after interoceptive compared with exteroceptive awareness (Study 1). The second aim was to investigate the relationship between interoceptive awareness, alexithymia and their association with glutamate and GABA concentrations in insula and ACC (Study 2).

2 Empirical part

2.1 Overview

Study 1 Interoceptive Awareness Enhances Neural Activity During Empathy

Published in Human Brain Mapping 34:1615-1624 (2013)

Study 2 The association of interoceptive awareness and alexithymia with neurotransmitter concentrations in insula and anterior cingulate

Published in Soc Cogn Affect Neurosci doi: 10.1093/scan/nst058 (2013)

2.2 Study 1 Interoceptive Awareness Enhances Neural Activity During Empathy

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Key words: fMRI; empathy; interoceptive awareness

Abstract

Empathy is a multicomponent function that includes sensorimotor, affective, and cognitive components. Although especially the affective component may implicate interoception and interoceptive awareness, the impact of interoception on empathy has never been evaluated behaviorally or neurophysiologically.

Here, we tested how a preceding period of interoceptive awareness impacts and modulates neural activity during subsequent empathy. We used functional magnetic resonance imaging (fMRI) and measured the sequential interaction between interoception and empathy using fMRI in 18 healthy subjects. We found that the preceding interoceptive awareness period significantly enhanced neural activity during empathy in bilateral anterior insula and various cortical midline regions. The enhancement of neural activity during empathy in both interoceptive and empathy networks by preceding interoceptive awareness suggests a close relationship between interoception and empathy; thereby, interoception seems to be implicated to yielding empathy.

Introduction

Empathy, a phenomenon characterizing our understanding and sharing of others' feelings, is vital to everyday communication and survival in a social environment (Eisenberg and Strayer, 1987) and can be broadly defined as the experiencing of an affective or sensory state similar to that shown by a perceived individual, where one is aware as to whether the source of the state is oneself or another (Batson, 2009). Empathy consists of both automatic affective experience and controlled cognitive processing, which are distinct but interrelated processes that may be instantiated differently in the brain (Decety and Jackson, 2004; Keysers and Gazzola, 2007; Singer, 2006; Watt, 2007). Animal data suggest that maternal care and nurturance might reflect a kind of protoempathy (Panksepp, 1998) and might be phylogenetically coincident with the social bonds (Watt, 2007). Such social bonding is highly

relevant for an evolutionary consideration of empathy, as it has been pointed out by the hallmark work of Panksepp (1998) and Watt (2007). Empathy thus needs to be considered within a socioevolutionary context meaning that empathic abilities are essential to the capacity to have stable attachments, with social bonding being critically enhanced by the ability to perceive the distress of a conspecific. This is being supported by imaging studies in humans investigating attachment in maternal care and romantic love and revealing a large functional overlap with regions of the empathy network (Bartels and Zeki, 2000, 2004; Lorberbaum et al., 2004). Diminished empathic abilities and unstable or nonexistent relationships as evident in autism, sociopathy, and borderline personality disorder also suggest close ties between attachment and empathy (Baron- Cohen, 2010; Dziobek et al., 2011; Frick and White, 2008; Shamay-Tsoory et al., 2010; Watt, 2007). Empathy studies in different domains such as pain, touch, and disgust (Wicker et al., 2003; Morrison et al., 2004; Singer et al., 2004; Jackson, Meltzoff, and Decety 2005) have yielded a quite consistent neural network that comprises the bilateral anterior insula, the anterior cingulate cortex, the thalamus, and the medial prefrontal cortex (Fan et al., 2010, 2011; Molenberghs et al., 2011; Singer and Lamm, 2009; Decety et al., 2006; Lamm et al., 2007, 2011) that is activated during the observation as well as during the experience of the respective sensations. The simulation theory of empathy therefore proposes that humans understand the thoughts and feelings of others by using their own mind as a model. By simulating the experience of another person in our own mind, we can intuitively understand what that experience might be like (Gordon, 1986). The discovery of mirror neurons and other ‘‘shared circuits’’ that are commonly activated by one’s own and another’s actions have been viewed as neural evidence in support of simulation theory (Gallese and Goldman, 1998; Rizzalotti, 2010). Nonconscious neural mirroring may allow for the vicarious experience of the emotional states of others and enable the affective sharing characteristic of empathy (Decety and Jackson, 2004; Gallese, 2003; Iacoboni et al., 1999). This idea has been supported by studies showing that imitation and observation of emotional facial expressions, which commonly activates mirror neuron and limbic regions with the insula

as a relay station for transmitting action information from premotor mirror areas to limbic areas, which then process emotional content (Carr et al., 2003). Although the role of mirror neurons in empathy has been questioned by some authors (see Watt, 2007), there is consistent and strong evidence for their involvement in the affective component of empathy, specifically in emotional contagion. It has been suggested that overt facial mimicry is related to emotional contagion (Keysers and Gazzola, 2006; Niedenthal 2007; Jabbi et al., 2007; Schulte-Ruther et al., 2007; Nummenmaa et al., 2008; Shamay-Tsoory et al., 2009). Because especially affective states are often assumed to involve awareness of one's own bodily state, processing of bodily and thus interoceptive stimuli may be a crucial component in yielding empathy (Northoff, 2007). This is supported by a recent electroencephalographic study that demonstrated the variation of the heartbeat-evoked potential (as cortical electrophysiological measure of interoception) during empathy (Fukushima et al., 2011). Although this study demonstrated the dependence of interoception on empathy, it though remains unclear how the neuronal processes during empathy are modulated by interoception or more specifically during interoceptive awareness. The assumption of a close relationship between interoceptive awareness and empathy is further supported when considering the regions and neural networks recruited during both processes. Interoceptive awareness has been investigated by using a visual or auditory heartbeat feedback with the subjects' task being a synchronicity judgement about this feedback (Critchley et al., 2004; Matthias et al., 2009; Pollatos et al., 2005, 2007). Wiebking et al. (2010, 2011) applied an interoception paradigm where subjects were asked to silently count their own heartbeat for as long as a task-type indicator was displayed. Interoceptive awareness leads to neural activity changes in the bilateral anterior insula, the anterior cingulate cortex, and the thalamus (Critchley et al., 2004, 2010; Wiebking et al., 2010, 2011). Taken together, these results of a considerable regional overlap between empathy and interoception suggest functional interdependence. Analogously to previous studies in the visual (Kastner et al., 1999) and emotional (Bermpohl et al., 2006; Grimm et al., 2006) domains, we applied a sequential interaction design where the empathy period was

preceded by periods of intero- or exteroceptive awareness. Although most previous empathy studies mainly focused on sensory qualities, and subjects were not instructed to engage (or not engage) in empathic processing, we applied an empathy task that required subjects to make empathy judgements for facial expressions and therefore specifically asked them to engage in empathic processing (Fan et al., 2011; deGreck et al., 2011). Even though this might seem similar to an emotion recognition task (Matsumuto et al., 2000; Jehna et al., 2011), the crucial difference is the requirement of an explicit empathy judgement rather than an emotion classification. The first aim of the study was to investigate how empathy related neural activity in the interoceptive network is modulated by preceding interoceptive awareness. We hypothesized that the neural activity during empathy in these regions is enhanced by preceding interoceptive awareness (when compared with empathy preceded by exteroceptive awareness or empathy without any preceding awareness). Second, we aimed to investigate whether regions of the empathy network are differentially modulated during empathy after interoceptive compared with exteroceptive awareness. We hypothesized that the preceding interoceptive awareness would significantly enhance neural activity during empathy in regions of the empathy network.

Material and Methods

Participants

Healthy subjects (12 women and 6 men, mean age 27 (SD 7.6)) were recruited from online study advertisements. Exclusion criteria were major medical illnesses, histories of seizures, head trauma with loss of consciousness, and pregnancy. In addition, subjects who met criteria for any psychiatric or neurologic disorder had a history of substance abuse in the previous six months or had a history of substance dependence were excluded from the study. All subjects were right-handed as assessed with the Edinburgh Handedness Inventory (Oldfield et al.,

1971). The study was carried out in accordance with the latest version of the Declaration of Helsinki and approved by the State of Zurichs' Review Board. All subjects gave written informed consent before screening.

Pictorial Stimuli

Participants viewed full-color pictures of the Japanese and Caucasian Facial Expressions of Emotions (JACFEE, Matsumoto and Ekman, 1988) picture set. The set comprises 56 photos, including eight photos each of happiness, sadness, disgust, fear, surprise, anger, and contempt. Four photos of each emotion depict posers of either Japanese or Caucasian descent (two males, two females). Fourteen of the photographs were displayed twice in our paradigm, amounting to a total stimuli number of 70. Furthermore, the experiment included eight edited photographs from the JACFEE series with unrecognizable contents. Picture contents were transformed by using a smoothing function (Gerlach et al., 2002). The pictures were generated by Presentation VR (Neurobehavioral Systems, Albany, CA) and presented via video goggles (VisuaStim digital). Participants responded by pushing a fiber-optic light sensitive key press.

Experimental Design

The functional magnetic resonance imaging (fMRI) design was ‘‘event related’’ and based on a paradigm introduced by Critchley et al. (2004) where subjects had to attend to intero- and exteroceptive stimuli by counting their own heartbeat and tones. The original paradigm was altered by introducing an empathy condition, a control condition with blurred photographs (‘‘smooth’’), and rest periods. This modified paradigm has been successfully applied in two previous studies (Wiebking et al., 2010, 2011). During the interoceptive condition (‘‘interoception’’), subjects were asked to silently count their own heartbeat for as long as the task-type indicator (a black heart on a white background) was displayed (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 s). After each interoceptive task presentation, subjects were asked

to report the number of heartbeats counted via a simple visual scale (2 s). The marks on the scale represented the number of heartbeats subjects counted (<7, 7–12, 13–18, and >19). Subjects gave a response about the number of heartbeats by pressing one of the four buttons of the response box. This feedback component allowed subject's attendance to the task to be monitored. Exteroceptive conditions (''exteroception'') were indicated by a black musical note on a white background (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 s). During the task subjects had to silently count the number of tones heard during the period the task indicator was visible. To match the difficulty of the intero- and exteroceptive task, tones were presented at an individually determined, just audible volume. Furthermore, tones were presented constantly during the duration of the whole experiment, meaning also during Interoception. Analogous to the interoceptive condition, after each exteroceptive task, subjects reported the number of tones via button press (2 s). The marks on the scale represented the number of tones subjects counted (<18, 19–-25, 26–31, and >32). Tones were presented via headphones. For the empathy and control condition, subjects were presented original or smoothed photographs from the JACFEE series, respectively (see above). Each picture was presented for 4 s and had to be judged in both conditions regarding to whether subjects could empathize with the displayed emotion (yes–no option). Both conditions were either presented after the interoceptive and exteroceptive tasks (''EaI,'' Empathy after Interoception; ''EaE,'' Empathy after Exteroception; ''SaI,'' Smooth after Interoception; ''SaE,'' Smooth after Exteroception) or after the rest condition (''empathy'' and ''smooth''). Rest conditions were indicated by a black fixation cross on a white background (6.0, 6.5, 7.0, 7.5, and 8.0 s). Subjects were instructed to relax, fixate on the crosshair, and try to minimize all cognitive activity. The rest period allowed subjects to recover from the active tasks and, in addition, served as a baseline condition to distinguish between positive and negative BOLD responses (Stark and Squire, 2001). A total of 180 trials were presented in five runs; 25 trials were presented, respectively, for ''EaI,'' ''EaE,'' ''SaI,'' and ''SaE.'' Twenty trials were presented, respectively, for ''interoception,'' ''exteroception,'' ''empathy,'' and ''smooth''. The 70 photos of

the JACFEE series were presented within the ‘‘EaI,’’ ‘‘EaE,’’ and ‘‘empathy’’ trials. The eight different task conditions were pseudorandomized within and across the runs and their order counterbalanced across all subjects. Before the experimental session, the subjects were familiarized with the paradigm by completing a test run of eight trials.

Functional Imaging

Functional measurements were performed on a Philips Intera 3T whole-body MR unit equipped with an eightchannel Philips SENSE head coil. Functional time series were acquired with a sensitivity encoded (Pruessmann et al., 1999) single-shot echo-planar sequence (SENSEsshEPI). The following acquisition parameters were used in the fMRI protocol: echo time = 35 ms, field of view = 22 cm, acquisition matrix = 80 x 80, interpolated to 128 x 128, voxel size: 2.75 x 2.75 x 4 mm^3, and SENSE acceleration factor R = 2.0. Using a midsaggital scout image, 32 contiguous axial slices were placed along the anterior– posterior commissure plane covering the entire brain with a TR = 3000 ms ((q= 82°). The first three acquisitions were discarded due to T1 saturation effects.

Statistical Design

Behavioral Data

Reaction times were recorded during the fMRI measurement and analyzed in an univariate ANOVA. Given that the interoceptive/exteroceptive period and the empathy/ smooth period were associated with different tasks, response times were analyzed separately for the interoceptive/ exteroceptive periods and periods that required an empathy rating (empathy, smooth, EaI, EaE, SaI, and SaE). Data were analyzed using SPSS 16 (SPSS, 1989–2007).

fMRI Data

fMRI data were analyzed using MATLAB 6.5.1 (The Mathworks, Natick, MA) and SPM2 (Statistical Parametric Mapping software, SPM; Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl. ac.uk). Functional data were corrected for differences in slice acquisition time, realigned to the first volume, corrected for motion artefacts, mean adjusted by proportional scaling, normalized into standard stereotactic space (template provided by the Montreal Neurological Institute), and spatially smoothed using a 8-mm FWHM Gaussian kernel. The time series were high-pass filtered to eliminate low-frequency components (filter width 128 s) and adjusted for systematic differences across trials. Statistical analysis was performed by modeling the different conditions convolved with a hemodynamic response function as explanatory variables within the context of the general linear model on a voxel-by-voxel basis. Realignment parameters were included as additional regressors in the statistical model. A fixed-effect model at a single-subject level was performed to create images of parameter estimates, which were then used for a second-level randomeffects analysis. For the fMRI data group analysis, the contrast images from the analysis of the individual participants were analyzed using one-sample t tests. Clusters of activation were identified with a global height threshold of $P < 0.001$, uncorrected and a cluster threshold of greater than 5. fMRI analyses focused on the effect of the preceding intero- or exteroceptive condition on the empathy task. For the regions of interest (ROIs) analyses of peak voxels, coordinates which were obtained in contrasts of the group analyses (**Table 1**) were selected. ROIs were functionally defined by centering spheres on the respective peak voxels with a radius of 5 mm. Analyses were carried out for the bilateral insula (46, 6, 6; -42, 0, 6; x, y, z coordinates in MNI stereotactic space), precuneus (-4, -56, 28), posterior cingulate cortex (PCC bordering to the medial parietal cortex; -2, -10, 48), dorsomedial prefrontal cortex (DMPFC; 6, 44, 48) and supragenual anterior cingulate cortex (SACC; 10, 38, 26). For the ROI analyses, percent signal changes for the different conditions were extracted for each subject separately using Marsbar (http://marsbar. sourceforge.net/).

For each event, % signal changes were calculated relative to the mean signal intensity of this ROI across the whole experiment.

Results

Behavioral Data

During the fMRI experiment, there was neither a significant effect of the interoceptive/exteroceptive conditions ($F(2) = 0.609$, $P = .436$) nor of the conditions requiring an empathy rating ($F(2) = 1.108$, $P = .234$) on reaction times. Inclusion of age and sex as co-variates did not have any influence on the results.

fMRI Data

Enhancement of empathy-related activity in interoceptive network by preceding interoception

For the contrast interoception > exteroception we found larger signal intensities in the bilateral insula (see **Table 1** and **Fig. 1**). Pursuing a ROI approach, we then calculated the signal changes for the various empathy conditions. This yielded stronger signal changes in bilateral insula during empathy preceded by interoceptive awareness when compared with empathy following either exteroceptive awareness (left insula: $P = .021$; right insula: $P = .056$) or no awareness (right insula: $P = .021$; see **Fig. 1**). Interestingly, the signal changes during EaI were even stronger than those during interoception alone (see **Fig. 1**), even though these differences were not significant.

Enhancement of activity during EaI when compared with empathy following exteroception

After searching for signal changes in the interoceptive network, we focused on those regions that were significantly stronger activated during EaI when compared with EaE. This contrast (EaI > EaE) yielded significant signal changes in the SACC, the DMPFC, the PCC (bordering to the medial parietal cortex), and the precuneus (see **Table 1** and **Fig. 2a,b**). The region-of-

interest based analysis demonstrated stronger signal changes during EaI when compared with empathy alone (SACC: P = .008; DMPFC: P = .032) (see **Fig. 2a**)).

Region	Side	EaI > EaE	Interoception > Exteroception	Empathy > Smooth
Insula	R		46 6 6 z: 3.87	32 18 8 z: 3.63
	L		-42 0 6 z: 4.33	-28 32 -8 z: 4.94
Precuneus	L	-4 -56 28 z: 3.25		
PCC (bordering MPC)	L	-2 -10 48 z: 3.39		
DMPFC	R	6 44 48 z: 3.63		2 18 48 z: 5.77
SACC	R	10 38 26 z: 3.58		
Middle temporal Gyrus	L	-54 -28 -16 z: 3.87		
MPC	R/ L	8 -40 74 z: 3.25		
Amygdala	R			16 -2 -20 z: 4.34
Thalamus	L			-8 -6 12 z: 4.38

Table 1 :Summary of brain regions significantly activated during the various conditions.

EaI, Empathy after Interoception; EaE, Empathy after Exteroception; PCC, posterior cingulate cortex; DMPFC, dorsomedial prefrontal cortex; SACC, supragenual anterior cingulate cortex; MPC, medial parietal cortex. The global height threshold was set to P < 0.001 uncorrected, the extent threshold to k = 5 voxels for all contrasts. The values in the table represent maximum z values with peak voxel coordinates in the MNI stereotactic space.

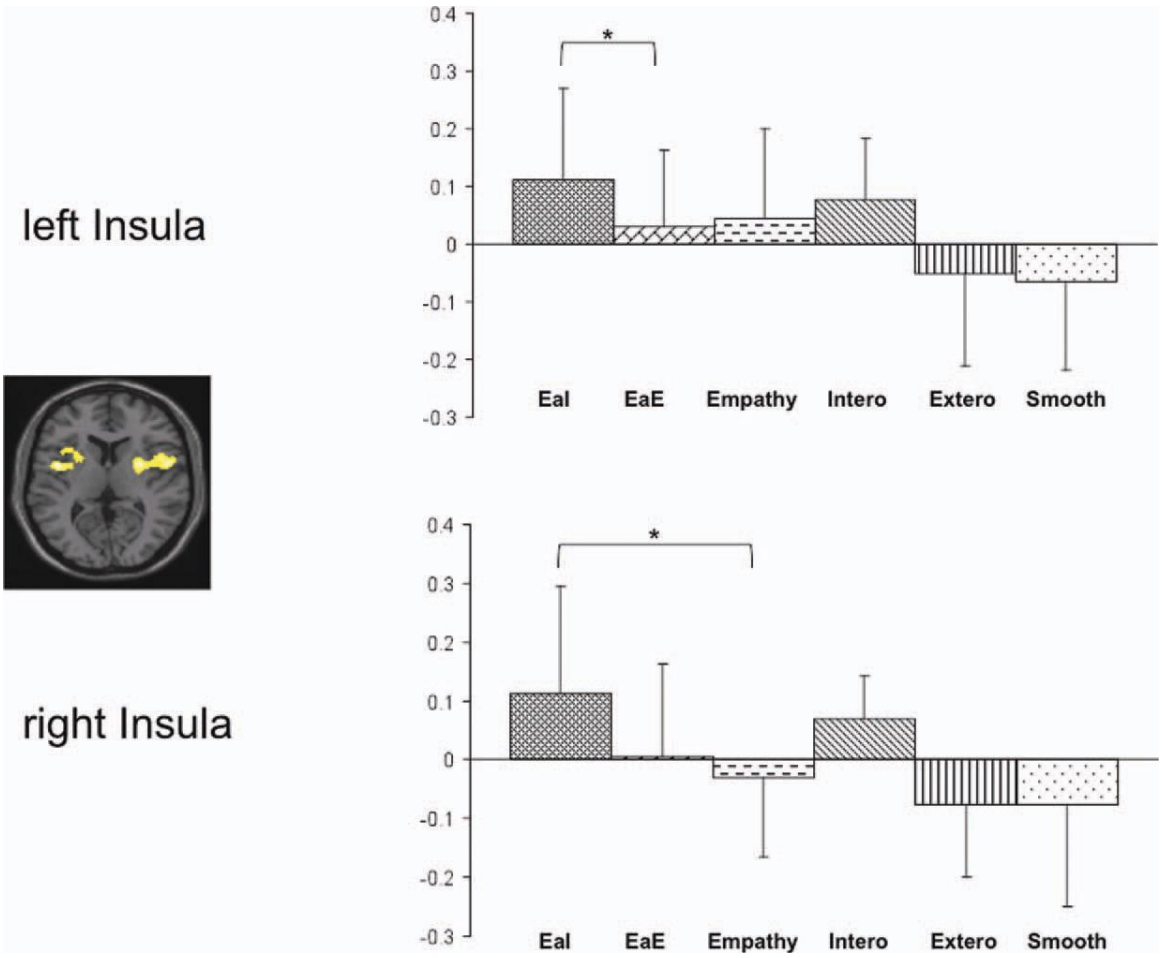


Figure 1: Signal changes in interoceptive regions.

The SPM image shows the statistical parametric (T) map for the contrast interoception > exteroception, overlaid on a single subject's normalized brain in the MNI stereotactic space (*P* < 0.001; uncorrected; *k* > 5).

Bar diagrams show % signal changes during EaI, EaE, empathy, interoception, exteroception, and smooth in the bilateral insula (46, 6, 6; -42, 0, 6). Abbreviations: EaI, Empathy after Interoception; EaE, Empathy after Exteroception; Intero, interoception.

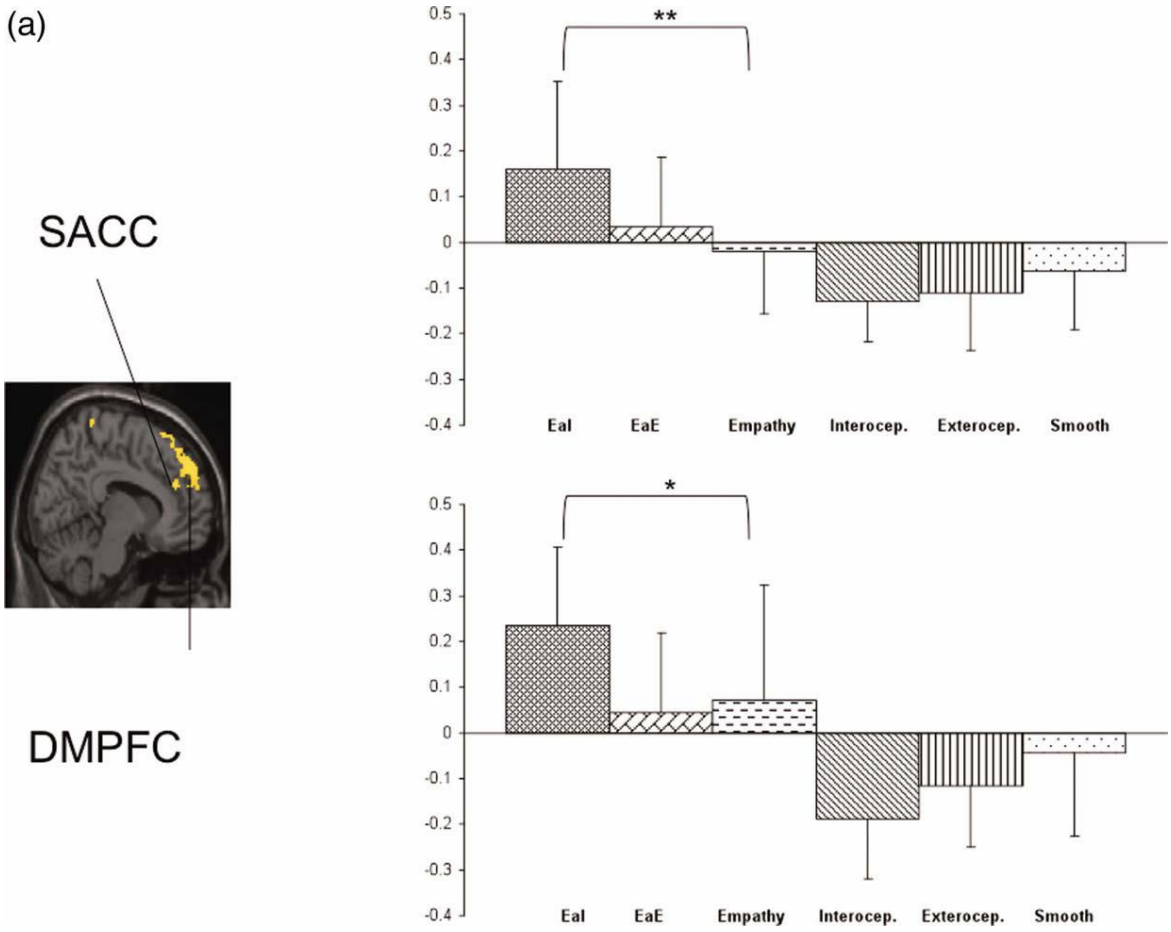
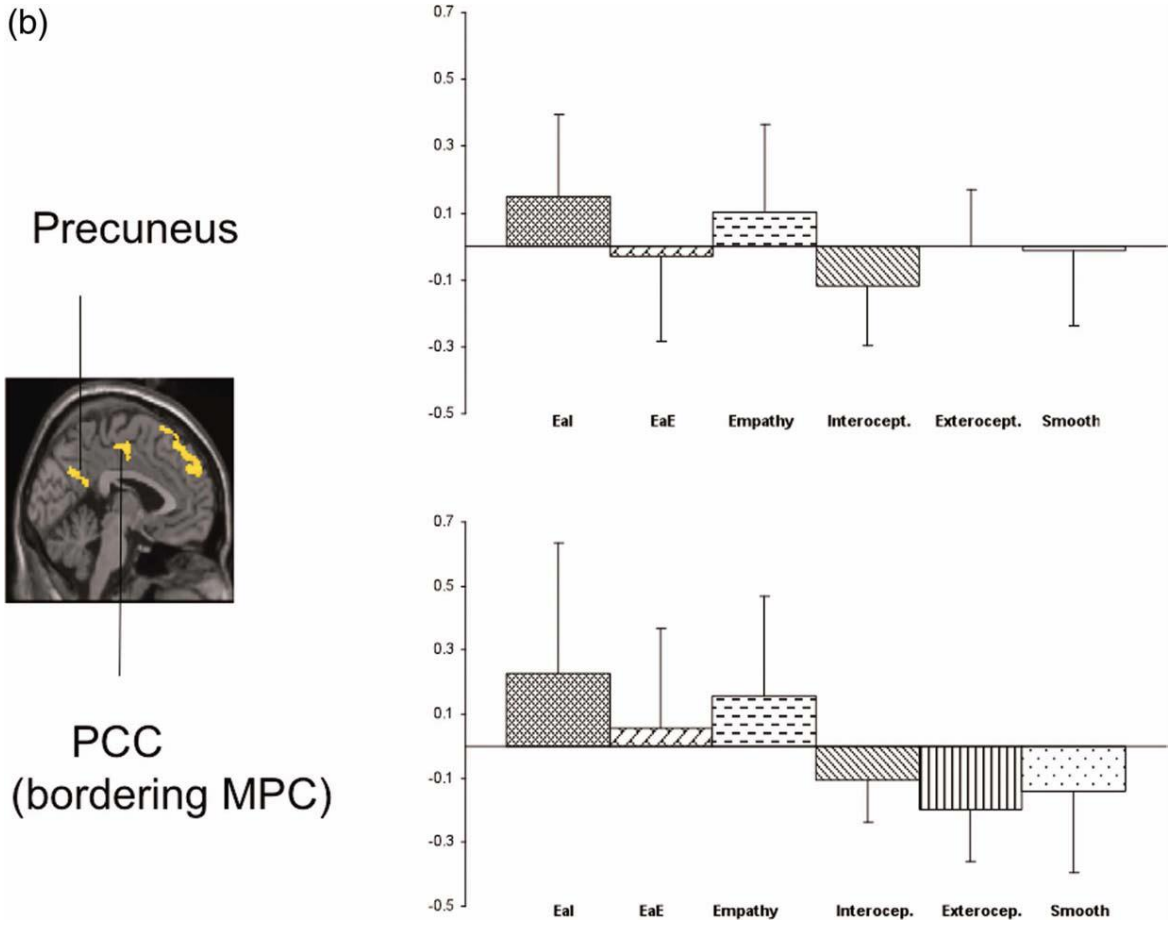


Figure 2 a,b: Signal changes in empathy regions

(**a**) SPM image shows statistical parametric (T) map for the contrast EaI > EaE, overlaid on a single subject's normalized brain in the MNI stereotactic space ($P < 0.001$; uncorrected; $k > 5$). Bar diagrams show % signal changes in EaI, EaE, empathy, interoception, exteroception, and smooth. Bar diagrams show % signal changes in the SACC (10, 38, 26) and DMPFC (6, 44, 48).



(**b**) SPM image shows statistical parametric (T) map for the contrast EaI > EaE, overlaid on a single subject's normalized brain in the MNI stereotactic space ($P < 0.001$; uncorrected; $k > 5$). Bar diagrams show % signal changes in EaI, EaE, empathy, interoception, exteroception, and smooth. Bar diagrams show % signal changes in the Precuneus (-4, -56, 28) and PCC (bordering to the medial parietal cortex) (-2, -10, 48). Abbreviations: EaI, Empathy after Interoception; EaE, Empathy after Exteroception; Intero, interoception; Extero, exteroception; SACC, supragenual anterior cingulate cortex; DMPFC, dorsomedial prefrontal cortex; PCC, posteriorcingulate cortex; MPC, medial parietal cortex. *: $P < 0.005$; **: $P < 0.001$.

Discussion

Here, we investigated the relationship between interoception and empathy. Our first main finding is that a preceding interoceptive awareness period significantly enhances neural activity during empathy in those regions recruited during interoception, i.e., bilateral anterior insula. The second main finding is that preceding interoceptive awareness enhances neural activity during empathy in anterior and posterior midline regions like the SACC, DMPFC, PCC, and precuneus. Most importantly, in interoceptive and empathy-related regions, signal changes during EaI were even stronger than those during interoception and empathy alone. Taken together, our findings indicate enhancement of neural activity during empathy in both interoceptive and empathy networks by preceding interoceptive awareness. This suggests a close relationship between interoception and empathy with the former being apparently implicated in yielding the latter. Our first main finding concerns the modulation of empathy-related neural activity in interoceptive regions. Although many studies indicated recruitment of the bilateral anterior insula in both empathy (Fan et al., 2011) and interoception (Critchley et al., 2004, 2005; Wiebking et al., 2010; Craig, 2002, 2009, 2010; Paulus et al., 2007), the relationship between interoception and empathy has not been studied so far. Our results demonstrate that the neural activity in the bilateral anterior insula during empathy can be significantly enhanced by preceding interoception. More specifically, preceding interoceptive awareness enhanced neural activity during subsequent empathy when compared with either empathy with exteroceptive awareness or no preceding awareness period at all. This suggests a specific interaction of empathy with interoception, i.e., interoceptive awareness, as distinguished from exteroceptive awareness. What does our finding imply for empathy? Empathy is considered to consist of several components including sensorimotor, affective, and cognitive functions (Fan et al., 2010; Singer and Lamm, 2009; Decety et al., 2006; Lamm et al., 2007, 2010, 2011; Schnell et al., 2011). The observation of emotional facial expressions commonly activates mirror neuron and limbic regions with the insula as a relay station. This nonconscious neural mirroring may allow for emotional contagion (Watt, 2007;

Shamay-Tsoory, 2011) as well as for affective sharing (Decety and Jackson, 2004; Gallese, 2003; Iacoboni et al., 1999) as discussed in the simulation theory of empathy (Gordon, 1986; Gallese and Goldman, 1998). Our study highlights the central relevance of interoceptive function. More specifically, one may be inclined to assume that the here observed enhancement of empathy-related activity in the bilateral insula by the preceding interoception indicates the implication of interoceptive function in empathy itself. Only when interoception is implicated in empathy itself, the preceding interoceptive awareness period can exert such strong enhancement effects as observed here. This is further supported by the fact that empathy following the interoceptive awareness induced stronger activity not only than the two other empathy conditions but was also stronger than interoception alone. Hence, one may be inclined to assume that the preceding interoceptive period enhances an already existing interoceptive component in empathy itself. Because the insula and especially the anterior insula is often assumed to integrate different stimuli especially interoceptive ones (see Craig, 2002, 2009, 2010; Paulus and Stein, 2010) one may consequently assume a process we call intero–intero interaction underlying the enhancement of bilateral anterior insula activity. In addition to the bilateral anterior insula, other regions also showed enhancement of empathy-related neural activity by preceding interoceptive awareness. This concerned especially anterior and posterior midline regions like the SACC, DMPFC, PCC, and precuneus. In addition to empathy (Fan et al., 2010), these regions have often been implicated in self-related processing, a process where stimuli are related to the own person (see Northoff et al., 2006, Qin and Northoff, 2010). Because interoceptive stimuli stem from the own body, they may show a rather high degree of self-relatedness which in turn may account for the enhancement of neural activity in these regions by the preceding interoceptive awareness period. This however remains speculative at this point and needs to be addressed in future studies testing for the interaction between empathy, interoception, and self-relatedness. Finally, given the supposedly central role of interoception in empathy, one may also need to reconsider empathy. Although the role of exteroceptive stimuli as coming from the other

person one shows empathy with has been highlighted, the role of the interoceptive stimuli stemming from the own body remains unclear. Following our results, one may be inclined to argue that empathy may be regarded as a special form of linkage between intero- and exteroceptive stimuli thus presupposing what one may want to call intero–extero interaction. Although plenty of studies have investigated how empathy and its underlying neural activity depend on exteroceptive stimuli and the exteroceptive context (Fan et al., 2010; Singer and Lamm, 2009; Decety et al., 2006; Lamm et al., 2007, 2010, 2011; Schnell et al., 2011), no study demonstrated how the variation of interoceptive stimuli impacts empathy. On the basis of our results shown here one would hypothesize that variation of the interoceptive state of one's body also impacts the degree of neural activity and possibly the behavioral manifestation of empathy itself. This though remains to be demonstrated in the future. Some methodological limitations need to be mentioned. First, we did not include the reverse testing of empathy modulating interoception. Future studies may want to investigate this relationship to further and better understand how interoception is implicated in empathy. Second, one needs to distinguish between interoception and interoceptive awareness with the former not necessarily entailing the latter. In our study, we targeted interoceptive awareness rather than mere interoceptive processing per se. Future designs may want to investigate whether both exert differential effects on empathy including its distinct components, sensorimotor, affective, and cognitive. Third, one may want to argue that the affective component of empathy already includes the interoceptive component with our study thus showing nothing new. However, unlike in previous studies, our approach explicitly isolated the interoceptive component and investigated its impact on subsequent empathy. Future studies may therefore want to focus especially on the interaction between interoception and affect within empathy. Fourth, future studies should include an empathy questionnaire such as the Balanced Emotional Empathy Scale (Mehrabian, 1996) to provide an empathy ‘‘trait’’ score and allow for further investigation of the relationship between empathic abilities and specific neural activation patterns. Fifth, one might argue that interoception may have a general facilitative relationship

to all forms of affect and affective experience but not a specific relationship to empathy per se. Future studies should investigate the impact of interoceptive awareness on other forms of emotional processing to answer this critical question. Lastly, one also needs to consider possible attention differences between the interoceptive and exteroceptive awareness conditions as well as carry-over effects of these preceding conditions on the empathy condition. Because preceding exteroceptive awareness would not only be expected to increase the attentional load to a comparable degree to interoceptive awareness and furthermore response times between these two conditions did not differ, attention differences can most likely be excluded. The same applies to carry-over effects, because the differential effect is not present yet during the preceding period of interoceptive versus exteroceptive awareness but only occurs during the subsequent empathy period. In conclusion, we here demonstrate for the first time a direct interaction between interoception and empathy. Our data show that preceding interoceptive awareness enhances neural activity in bilateral insula and various midline regions during empathy. Our data suggest the involvement of interoceptive processing in empathy which may interact with the exteroceptive stimuli in a specific way yielding what may be called intero–extero interaction. Though tentatively, this lets one to assume that the interoceptive components may need to be considered in empathy and added to the other various components.

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2.3 Study 2 The association between interoceptive awareness, alexithymia and neurotransmitter concentrations

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Abstract

Alexithymia and increased interoceptive awareness have been associated with affective disorders as well as with altered insula and anterior cingulated cortex (ACC) function. Brain imaging studies have demonstrated an association between neurotransmitter function and affective disorders as well as personality traits. Here, we first examined the relationship between alexithymic facets as assessed with the Toronto Alexithymia Scale (TAS-20) and interoceptive awareness (assessed with the Body Perception Questionnaire) in 18 healthy subjects. Second, we investigated their association with glutamate and gamma-aminobutyric acid (GABA) concentrations in the left insula and the ACC using 3-Tesla proton magnetic resonance spectroscopy. Behaviorally, we found a close association between alexithymia and interoceptive awareness. Furthermore, glutamate levels in the left insula were positively associated with both alexithymia and awareness of autonomic nervous system reactivity, while GABA concentrations in ACC were selectively associated with alexithymia. Although preliminary, our results suggest that increased glutamate-mediated excitatory transmission_related to enhanced insula activity_reflects increased interoceptive awareness in alexithymia. Suppression of the unspecific emotional arousal evoked by increased awareness f bodily responses in alexithymics might thus be reflected in decreased neuronal activity mediated by increased GABA concentration in ACC.

Introduction

The biological underpinnings of individual differences in personality traits are incompletely understood. Although there have been several functional imaging studies investigating the neuronal activity signatures of personality traits (Johnson et al., 1999; Canli et al., 2001, Canli and Amin, 2002; Canli, 2004; Kumari et al., 2004; Deckersbach et al., 2006; Vaidya et al., 2007; Simon et al., 2010; Brühl et al., 2011), less is known about their association with neurotransmitter concentrations. Specific personality traits such as low extraversion, high neuroticism (Watson and Clark, 1997; Kotov et al., 2010) and alexithymia (Luminet, 2010; Leweke et al., 2012) have been linked to increased vulnerability to psychiatric disorders, specifically affective disorders, which are in turn associated with dysfunctional neurotransmission (Mathew et al., 2008; Sanacora et al., 2008; Walter et al., 2009; Hashimoto et al., 2010; Grimm et al., 2012a). Of specific relevance with regard to affective disorders are findings of a negative correlation between prefrontal glutamate (Glu) concentrations, mental perspective taking and extraversion (Montag et al., 2008; Grimm et al., 2012b). In addition, decreased and increased ACC gamma-aminobutyric acid (GABA) concentrations, respectively, have been associated with extraversion and harm avoidance (Kim et al., 2009; Goto et al., 2010) in healthy subjects. Thus, the investigation of alexithymia might be promising, since it is marked by cognitive and affective features including difficulties in identifying and describing feelings as well as in distinguishing feelings from bodily sensations of emotional arousal (Franz et al., 2008). Alexithymics can be characterized by low extraversion, high neuroticism, high harm avoidance, low selfdirectedness and low perspective taking (Wise et al., 1992; Guttman and Laporte, 2002; Picardi et al., 2005). This personality trait is prevalent in 10% of the general population (Linden et al., 1995; Salminen et al., 1999) and its facets have been identified as a risk factor for affective disorders (Conrad et al., 2009; Luminet, 2010; Leweke et al., 2012). Results from neuroimaging studies indicate a crucial role of insula and ACC in mediating alexithymic features. In both regions, heterogeneous findings with either increased (Berthoz et al., 2002; Mériau et al., 2006;

Frewen et al., 2008; Karlsson et al., 2008; Heinzel et al., 2010) or decreased (Leweke et al., 2004; Karlsson et al., 2008; Silani et al., 2008; Bird et al., 2010; Reker et al., 2010) response to emotion stimuli have been reported in alexithymic individuals. Data on structural changes in alexithymia are inconsistent, with studies reporting a correlation between alexithymia and the size of the right ACC (Gu¨ndel et al., 2004), smaller volumes of ACC, medial temporal gyrus and anterior insula in alexithymic women (Borsci et al., 2009) as well as no volume difference in alexithymic men (Heinzel et al., 2012). Reduced gray matter volume in the ACC has been recently associated with an interaction between two polymorphisms on the BDNF and DRD2/ANKK1 gene (Montag et al., 2010) which in turn are also associated with alexithymia (Walter et al., 2011). Insula and ACC are implicated in processing of affect, self-awareness and mood and show functional alterations in affective disorders (Bush et al., 2000; Mayberg, 2003; Phillips et al., 2003; Grimm et al., 2009, 2011; Horn et al., 2010; Wiebking et al., 2010). A recently proposed model by Medford and Critchley (2010) states that the conjoint activity of insula and ACC is crucial for the production of subjective feelings and co-ordinating appropriate responses to internal and external stimuli, thereby providing the neural basis of self-awareness. The insula also has a central role in attention to interoceptive states (Critchley et al., 2004; Pollatos et al., 2007; Menon and Uddin, 2010; Terasawa et al., 2011; Simmons et al., 2012). Interoceptive awareness at least partially mediates emotional experience (Bechara et al., 1996; Pollatos et al., 2005; Werner et al., 2009; Dunn et al., 2010) and the degree of interoceptive awareness might modulate the emotional experience (Wiens and Palmer, 2001; Critchley et al., 2004; Werner et al., 2009). In affective disorders, studies have reported alterations in interoceptive awareness and insula activity (Wiebking et al., 2011). Furthermore, glutamate concentrations in ACC predict the restingstate functional connectivity between insula and ACC in depressed patients (Horn et al., 2010). The ACC is not only crucial for emotion processing in general (Bush et al., 2000; Kober et al., 2008) but also for constituting our sense of self and consecutively for becoming aware of one's self (McKiernan et al., 2006; Northoff et al., 2006). A recent study by Liemburg et al. (2012)

reported lower connectivity in anterior cortical midline structures including the ACC in alexithymic subjects. Functional alterations, as reflected in reduced negative BOLD responses in these regions during emotional and self-related processing, have been associated with increased self-focus of depressive patients (Grimm et al., 2009, 2011). Negative BOLD responses in ACC are mediated by GABA in healthy subjects (Northoff et al., 2007), but by glutamate in depressive patients (Walter et al., 2009). Despite the specific role of both insula and ACC in alexithymia as well as in interoceptive awareness and the behavioral relevance of altered neurotransmission in these regions, the respective relationships have not been elucidated yet. Therefore, we first aimed to investigate the association of interoceptive awareness with alexithymic features. Second, we aimed to examine the association between alexithymic features, interoceptive awareness and glutamate and GABA concentrations in the left insula and ACC of healthy subjects. We hypothesized a relationship between interoceptive awareness and alexithymic features. For the insula, we predicted an association between increased interoceptive awareness, alexithymic features and glutamate concentration. Based on reports of alterations in ACC responses in alexithymia (Berthoz et al., 2002; Karlsson et al., 2008; Heinzel et al., 2010) and findings showing that ACC signal changes are mediated by GABA (Northoff et al., 2007), we predicted alexithymic features to be associated with GABA concentrations in ACC.

Material and Methods

Subjects

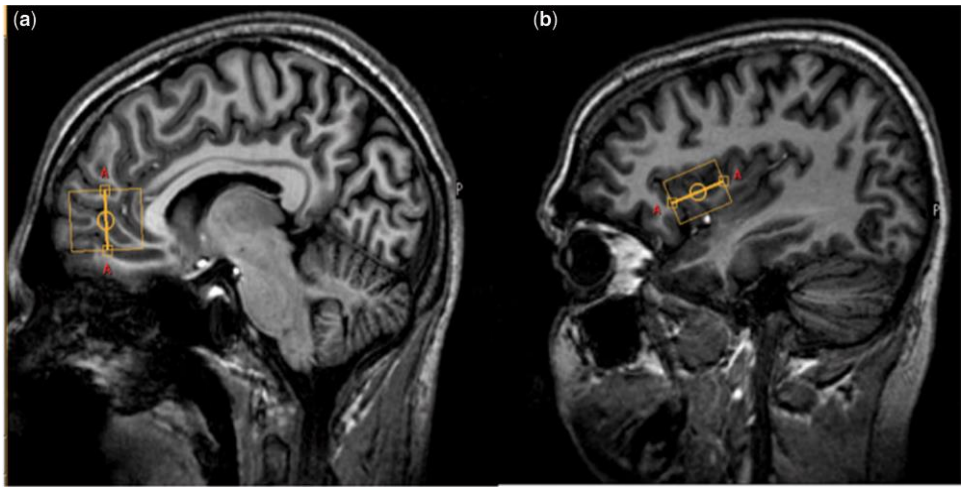
Healthy subjects (13 women and 9 men, mean age 27.12 (s.d. 7.6)) were recruited through online study advertisements. Exclusion criteria were major medical illnesses, histories of seizures, head trauma with loss of consciousness and pregnancy. In addition, subjects who met criteria for any psychiatric or neurologic disorder or had a history of substance dependence were excluded from the study. All subjects were right-handed as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). The study was performed in

accordance with the latest version of the Declaration of Helsinki and approved by the State of Zurichs' Review Board. All subjects gave written informed consent. Subjects were investigated with proton magnetic resonance spectroscopy (1H-MRS), the 20-item Toronto Alexithymia Scale (TAS-20; Bagby et al., 1994a, German version by Bach et al., 1996) and the Body Perception Questionnaire (BPQ; Porges, 1993). The TAS-20 is a self-administered questionnaire that captures two affective and one cognitive alexithymic facets, respectively: difEculties identifying feelings (e.g. I am often confused about what emotion I am feeling), difEculties describing feelings (DDF, e.g. it is difficult for me to find the right words for my feelings) and a concrete, externally oriented thinking (EOT) style (e.g. being in touch with emotion is essential; inverted item). The scale has a good psychometric quality (Cronbach's $\alpha > 0.80$; Bagby et al., 1994b) and is widely used in emotion research, so that comparability with previous studies is assured. Its psychometric properties have previously been investigated in healthy subjects (Franz et al., 2008). Each item of the TAS-20 is rated on a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The BPQ (Porges, 1993) is a 96 item self-report instrument to assess body perception and interoceptive awareness on four subscales (awareness subscale: subjects are asked to imagine how aware they are of their bodily processes (e.g. swallowing frequently); stress response: subjects are asked to imagine being in a very stressful situation and rate their bodily changes due to that situation (e.g. emotional problems such as more frequent feelings of depression, frustration, rage or anger); autonomic nervous system reactivity: requires that subjects answer items about their own autonomous nervous system reactions (e.g. 'my heart often beats irregularly'); stress style subscale: evaluates the manner in which the subject responds to stress (e.g. 'I have difficulty speaking')). Each item is rated on a five-point Likert scale ranging from 1 (never) to 5 (always).

Spectroscopic data acquisition and analysis

Single voxel 1H-MRS data were acquired at rest from two volumes of interest (VOI) in each subject using a Philips Achieva 3-T whole-body MR unit (Philips Medical Systems, Best, The Netherlands) equipped with a birdcage transmit-receive head coil. One VOI of 32x21x24mm^3 = 16.128 ml was placed in the left insula, while a second one (25x25x25mm^3 = 15.625 ml) was placed in the ACC (**Figure 1**). To enable an unambiguous measurement of metabolites, data from each VOI were acquired using a 2D JPRESS sequence (Schulte and Boesiger, 2006), which encodes the J coupling along the indirect spectral dimension by acquiring data with multiple echo times. This approach allows for a significant reduction of spectral overlap by spreading multiplet resonances along two frequency axes. The sequence was preceded by water suppression using frequency-selective excitation and gradient spoiling followed by adiabatic frequency-selective rephasing and gradient spoiling. The echo times for the JPRESS experiment ranged from 28 to 228 ms with a step size of 2 ms and a phase cycling of 16 for each TE. Other parameters included a bandwidth in the direct dimension of 2 kHz and 2048 sample points. Using 100 encoding steps and eight averages per encoding step at a repetition time of TR = 2000 ms, the acquisition time for one voxel accounted to 24 min. JPRESS data were quantified using ProFit (Schulte and Boesiger, 2006), a two-dimensional fitting procedure, which applies the full amount of prior knowledge by fitting a linear combination of simulated two-dimensional basis metabolite spectra. Simulation of the basis metabolite spectra was performed with GAMMA (Smith et al., 1994). Cramer-Rao lower bounds, an estimate of the fitting error, were used as a quality criterion to exclude data sets with unreliable quantification results. Hence, analyses were restricted to subjects who met strict quality criteria to indicate reliable spectral quantification (Cramer-Rao lower bounds 20%) for each metabolite and four subjects had to be excluded from the analysis. Because determination of absolute metabolite concentrations in millimolars requires a reliable T1 and T2 relaxation correction, while relaxation times of coupled metabolites are hardly known for

spectroscopy at 3-T, all metabolite concentrations are given relative to creatine levels. Creatine was proven to be an appropriate internal reference for the ProFit analysis (Schulte and Boesiger, 2006).



Placement of the magnetic resonance spectroscopic voxel (orange frame) in (a) the ACC and (b) the left insula.

Figure 1: Placement of the voxel

Statistical analyses

Statistical calculations were carried out as indicated in the 'Results' section using SPSS for Windows (Release 18.0; SPSS, Inc., Chicago, IL, USA). Within-group comparisons were performed using paired t-tests. Pearson correlation coefficients were computed to assess the relationship between neurotransmitter concentrations in both regions and the association between these concentrations and TAS-20 and BPQ scores. In an exploratory analysis, median split was used to create two groups of subjects with high and low scores on the TAS-20. Independent-sample t-tests were performed to analyze group differences between these subjects. Bonferroni corrections were used to counteract the problem of multiple comparisons. All tests were performed at a two-tailed level of significance of 5%.

Results

Behavioral data

TAS-20 and BPQ total scores and subscores are summarized in Table 1. The total score of the TAS-20 correlated significantly with the BPQ total score ($r = 0.70$, $P < 0.01$) as well as with the BPQ subscales for awareness ($r = 0.55$, $P < 0.05$), stress response ($r = 0.73$, $P < 0.05$), autonomic nervous system reactivity ($r = 0.65$, $P < 0.05$; **Figure 2**) and stress style ($r = 0.66$, $P < 0.05$). There were no effects of age and gender on TAS-20 and BPQ scores.

	Mean (SD; range)
TAS total Score	38.33 (7.88; 23- 51)
TAS- DIF Score	12.39 (5.03; 7-23)
TAS- DDF Score	10.61 (2.81; 6-17)
TAS- EOT Score	15.11 (3.55; 8-22)
BPQ total Score	172.83 (34.94; 117-223)
BPQ- Awareness Score	1.84 (.48; 1-2.56)
BPQ- Stress Response Score	2.12 (.62; 1.4-3.3)
BPQ- Autonomic Nervous System Reactivity Score	1.41 (.27; 1.07-1.93)
BPQ- Stress Style Score	2.53 (.33; 2.08-3)

Table 1: Questionnaire data

TAS, Toronto Alexithymia Scale; DIF, Difficulty Identifying Feelings; DDF, Difficulty Describing Feelings; EOT, Externally-Oriented Thinking; BPQ, Body Perception Questionaire.

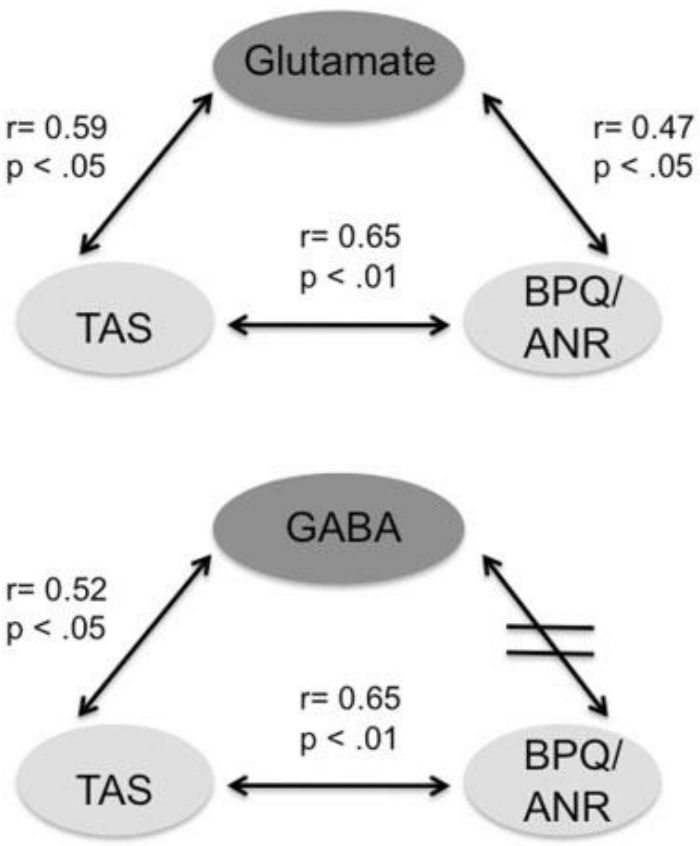
MRS data

First, concentrations of glutamate (Glu) and GABA did not differ significantly between the two investigated regions (Table 2). Glu concentrations in both regions were correlated ($r = 0.74$, $P < 0.01$), whereas GABA concentrations were not ($r = 0.33$, $P > 0.05$). Regarding the association of neurotransmitters with alexithymia and measures of sensitivity for bodily processes, we found a significant positive correlation between TAS total score and Glu concentrations in insula ($r = 0.59$, $P < 0.05$; **Figure 2**), which was mainly due to a strong correlation with the DDF ($r = 0.49$, $P < 0.05$) and EOT ($r = 0.53$, $P < 0.05$) subscores. Furthermore, there was a significant positive correlation between TAS total score and GABA concentrations in ACC ($r = 0.52$, $P < 0.05$; **Figure 2**), which was mainly due to a strong correlation with the DDF subscore ($r = 0.57$, $P < 0.05$). The BPQ subscore for autonomic nervous system reactivity also correlated with Glu concentration in insula ($r = 0.47$, $P < 0.05$), whereas no correlation was found with GABA concentrations in ACC (**Figure 2**). In an exploratory analysis, we investigated differences between subjects scoring high and low, respectively, on the TAS-20. High scoring subjects not only showed significantly higher BPQ scores (total score: $P < 0.01$; awareness score: $P < 0.05$; autonomic nervous system reactivity: $P < 0.01$) but also significantly higher Glu concentrations in insula, but not in ACC ($P < 0.05$). Strikingly, these subjects also showed a higher GABA concentration in ACC, but not in insula ($P < 0.07$; **Figure 3**).

	Left Insula	ACC
Glu/Cr	1.62 (±0.32)	1.59 (±0.26)
GABA/Cr	0.23 (±0.07)	0.25 (±0.06)

Table 2: Metabolite concentrations in insula and ACC

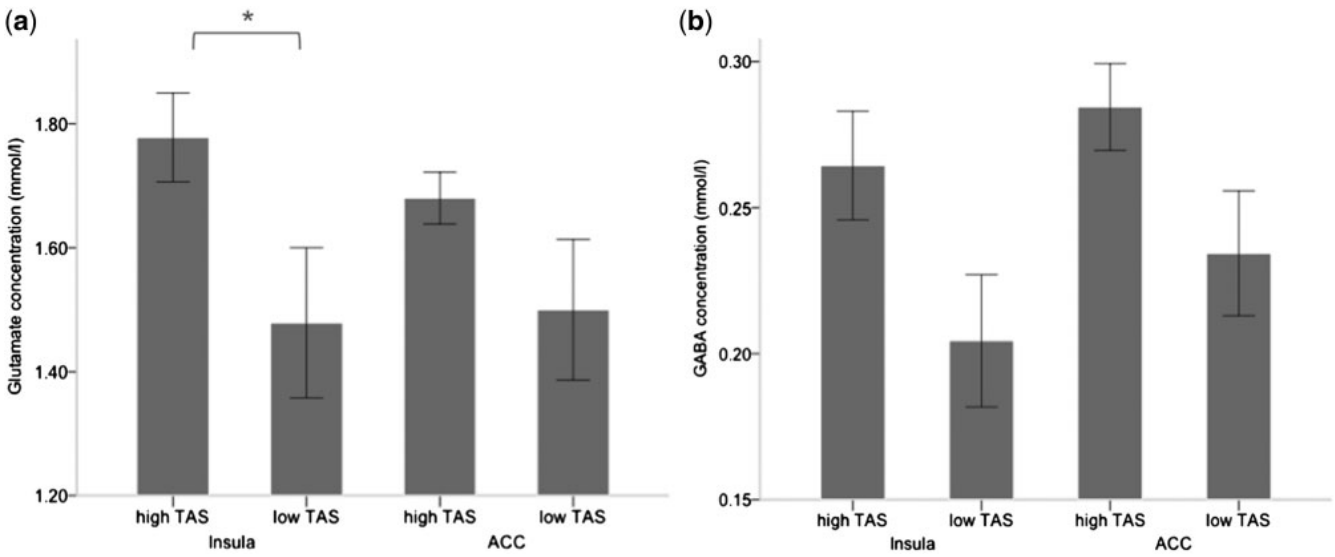
ACC, anterior cingulate cortex; Glu, glutamate; Gln, glutamine, GABA, Gamma-aminobutyric acid; NAA, N-Acetyl-Aspartat; CR, creatine



There is a significant correlation between alexithymia (TAS-20) and awareness of autonomic nervous system reactivity (BPQ-ANS). Glutamate levels in left insula are positively associated with both alexithymia and awareness of autonomic nervous system reactivity, whereas GABA concentrations in ACC are associated with alexithymia, but not with interoceptive awareness.

Figure 2: Schematic representation of correlations between functional (dark gray) and behavioral markers (light gray) in (a, above) the ACC and (b, below) the left insula.

TAS-20, Toronto Alexithymia Scale; BPQ, Body Perception Questionnaire; ANS, Awareness of Autonomous Nervous System Reactivity and ACC, anterior cingulate cortex.



Bar diagrams show (a) glutamate and (b) GABA concentrations in ACC and insula in subjects scoring high and low, respectively, on the TAS-20. Differences in glutamate concentration between these two groups were observed in insula ($P < 0.05$), but not ACC, whereas differences in GABA concentration were observed in ACC ($P < 0.07$), but not in insula. TAS-20, Toronto Alexithymia Scale; ACC, anterior cingulate cortex. *$P < 0.05$

Figure 3: Glutamate and (b) GABA concentrations in ACC and insula in subjects scoring high and low on the TAS-20

Bar diagrams show (a) glutamate and (b) GABA concentrations in ACC and insula in subjects scoring high and low, respectively, on the TAS-20. Differences in glutamate concentration between these two groups were observed in insula ($P < 0.05$), but not ACC, whereas differences in GABA concentration were observed in ACC ($P < 0.07$), but not in insula.
TAS-20, Toronto Alexithymia Scale; ACC, anterior cingulated cortex. *$P < 0.05$.

Discussion

The main goal of this study was to test the interconnection between alexithymic features, interoceptive awareness and concentrations of Glu and GABA in ACC and insula. As hypothesized, alexithymia was closely related to the different facets of interoceptive awareness. Glu levels in left insula and GABA concentrations in ACC were positively associated with alexithymia. Furthermore, there was a double dissociation of GABA and Glu concentrations in insula and ACC as a function of alexithymia: subjects scoring high on the TAS-20 showed high Glu concentration in insula, but not in ACC and high GABA concentration in ACC, but not in insula. Finally, Glu levels in left insula, but not in ACC, were positively associated with the awareness of autonomic nervous system reactivity. The difficulty in distinguishing feelings from bodily sensations of emotional arousal is a hallmark of alexithymia (Taylor, 2000). Even though previous data suggest a close association between alexithymia and altered interoceptive awareness, this was mainly concluded from the diminished insula response to emotional stimuli in alexithymics (Silani et al., 2008; Reker et al., 2010). Our results complement findings suggesting a significant relationship between interoceptive processes and subjective emotional experience (Wiens and Palmer, 2001; Critchley et al., 2004; Werner et al., 2009) by showing increased interoceptive awareness in subjects with more pronounced alexithymic features. However, we applied a subjective measure of body awareness (Porges, 1993), while previous studies often used interoceptive accuracy, as quantiEed by measuring an individual's ability to accurately perceive the own heartbeat (Cameron, 2001; Critchley et al., 2004; Pollatos et al., 2005) as an indicator for interoceptive awareness. Interoceptive accuracy is related to both insula volume and function as well as to subjective body awareness (Critchley et al., 2004). Specifically the awareness of autonomic system reactivity has been shown to predict interoceptive accuracy, which emphasizes the convergence between neurological and subjective traits associated with

interoception (Fairclough and Goodwin, 2007). A central role for insula in interoceptive awareness has been suggested by numerous neuroimaging studies (Critchley et al., 2004; Pollatos et al., 2007; Menon and Uddin, 2010; Terasawa et al., 2011; Simmons et al., 2012), whereas previous studies in alexithymia linked decreased insula response to reduced emotional awareness (Silani et al., 2008; Bird et al., 2010). However, these studies investigated the response to emotional stimuli, whereas interoceptive awareness, at least in healthy subjects, has been shown to increase insula activity (Critchley et al., 2004; Terasawa et al., 2011). Alexithymics show increased vulnerability to affective disorders, where studies have reported increased interoceptive awareness and insula activity, which might reflect the patients' inability to shift the focus of perception/awareness from the own body to the environment (Grimm et al., 2009; Wiebking et al., 2010). Our results show a close association between alexithymia and awareness of bodily and stress responses, which are in turn related to Glu concentration in insula. Elevated Glu levels in insula have been shown in acute and chronic pain and been discussed as an indicator for amplified interoceptive sensory processing (Harris et al., 2008; Gussew et al., 2010; Gutzeit et al., 2011), which is supported by our finding of a relationship between insula Glu concentration and awareness of autonomic nervous system reactivity. High interoceptive awareness, which in turn might be related to a Glu-mediated increase in insula activity, has been associated with higher emotional arousal (Wiens and Palmer, 2001; Pollatos et al., 2005). Likewise, the positive association reported between insula Glu concentration and alexithymia might reflect enhanced insula activity in alexithymia related to increased Glu-mediated excitatory transmission. The role of insula Glu in alexithymia is further emphasized by the region-specific elevation in Glu levels in subjects scoring high on the TAS-20. Thus, we propose that the interconnection observed between alexithymia, interoceptive awareness and Glu concentration in insula reflects increased awareness of bodily and stress responses as well as enhanced insula activity due to increased Glu-mediated excitatory transmission, which in turn might lead to a high unspecific arousal in alexithymic subjects. To the best of our knowledge, no studies have yet

investigated the modulation of alexithymic features by neurotransmitter concentrations. However, alexithymics show increased vulnerability to affective disorders (Luminet, 2010; Leweke et al., 2012), which are characterized by dysfunctional Glu and GABA-ergic neurotransmission (Sanacora et al., 2008; Mathew et al., 2008; Walter et al., 2009; Hashimoto et al., 2010; Grimm et al., 2012; Scheidegger et al., 2012). Furthermore, alexithymics show low extraversion, high neuroticism, high harm avoidance, low selfdirectedness and impaired perspective taking (Wise et al., 1992; Guttman and Laporte, 2002; Picardi et al., 2005). The correlation observed between Glu and alexithymic features is therefore well in accordance with previous studies investigating these traits and showing a negative correlation between prefrontal Glu and mental perspective taking as well as extraversion (Montag et al., 2008; Grimm et al., 2012b). Harm avoidance and extraversion have been associated with increased and decreased ACC GABA concentrations (Kim et al., 2009; Goto et al., 2010), respectively, which fits well with the reported correlation between ACC GABA and alexithymia. The ACC is a crucial region for emotion processing and for constituting our sense of self (McKiernan et al., 2006; Northoff et al., 2006). It has been hypothesized that while insula is involved in the generation of all subjective feeling states, combined action of insula and ACC might provide the neural basis of self-awareness (Craig, 2009). Medford and Critchley (2010) proposed that awareness of self, i.e. an integrated awareness of cognitive, affective and physical state is generated by the integrative functions of the insula and then re-represented in ACC as a basis for responses to inner or outer events. Back-projections from the ACC may then allow the insular representation of the feeling state to be modulated by cingulate activity. The proposed reciprocity between ACC and insula is supported by neuroanatomical studies (Nieuwenhuys et al., 2008; Moisset et al., 2010), findings of correlated BOLD signal fluctuations (Taylor et al., 2009; Horn et al., 2010) and joint activity of these areas during emotional experience (Harrison et al., 2008). Menon and Uddin (2010) characterize insula and ACC as a ‘salience network’ that functions to segregate the most relevant among internal and external stimuli in order to guide behavior. Insula activity modulates autonomic reactivity

to salient stimuli and Glu-mediated increased insula activity in alexithymia might reflect the misattribution of emotional salience to mundane events or bodily responses. A similar overdrive in the salience network has also been discussed in neuroticism, increased anxiety and depression (Paulus and Stein, 2006; Stein et al., 2007; Horn et al., 2010), all of which are closely related to alexithymia (Picardi et al., 2005; Luminet, 2010; Leweke et al., 2012). Our results show an association between GABA concentrations in ACC and alexithymic features. Since increased GABA transmission mediates a decrease in neuronal activity our finding is therefore well in accordance with previous studies showing lower ACC activation in response to emotion stimuli in alexithymia (Leweke et al., 2004; Moriguchi et al., 2007; Karlsson et al., 2008; Silani et al., 2008; Bird et al., 2010; Reker et al., 2010). Although we investigated Glu and GABA concentrations in the ventral ACC, a region crucially involved in emotional experience (Lane et al., 1998; Larisch et al., 1997; Bush et al., 2000; Northoff et al., 2007; Grimm et al., 2009), several previous studies reported increased activity in dorsal ACC in alexithymia (Berthoz et al., 2002; Me´riau et al., 2006; Frewen et al., 2008; Karlsson et al., 2008; Heinzel et al., 2010). The dorsal region of the ACC provides a cognitive processing of emotions (Bush et al., 2000; Beauregard et al., 2001) and is especially relevant for emotion regulation (Ochsner et al., 2002; Phan et al., 2005; Kim and Hamann, 2007; Wager et al., 2008). Alexithymia has been conceptualized as a disorder of emotion regulation (Swart et al., 2009), since it is associated with maladaptive coping strategies, notably emotional inhibition and immature defensive styles (Helmes et al., 2008). Increased activity in dorsal ACC might represent an effort to suppress the unspecific emotional arousal that results from increased interoceptive awareness and Glu-mediated enhanced insula activity (Swart et al., 2009; Heinzel et al., 2010) and eventually lead to GABA-mediated decreased activity in ventral ACC, which prevents excessive experience of negative emotions (Urry et al., 2009; Abler et al., 2010). Increased GABA concentrations in alexithymia might therefore indicate increased inhibitory control of ACC activity as a neuronal correlate of impoverished conscious experience of emotion in alexithymia (blindfeel) (Lane et al., 1997, 1998). This hypothesis is

supported first by the region-specific elevation in GABA levels in subjects scoring high on the TAS-20. Second, previous findings show that signal changes in ventral ACC during emotional processing are mediated by GABA (Northoff et al., 2007). Third, a recent study by Kupers et al. (2009) reports that acute pain and associated aversive emotional experience induced a significant increase in GABA in ACC. Finally, a recent study reported lower connectivity in ventral ACC in alexithymia (Liemburg et al., 2012). There are several limitations to this study. The rather small sample size has to be considered when interpreting the results. We did not control for phase effects within our female participants, which might be of importance since it has been demonstrated that luteal and follicular phases impact MRS metabolites (Batra et al., 2008). Additionally, although we investigated two regions related to alexithymia and interoceptive awareness, future studies should include a further control region and also investigate the right insula, since specifically right insula might support interoceptive awareness and integrate it with other information to form the basis of the subjective experience of an emotional state (Craig, 2003). Future studies should also consider including additional scales to shed further light on the association between other personality dimensions related to increased vulnerability to affective disorders (e.g. neuroticism) and neurotransmitter concentrations. In sum, this study indicates for the first time a close relationship between alexithymia, interoceptive awareness and GABA and Glu concentrations in ACC and insula. Increased Glu-mediated excitatory transmission and related enhanced insula activity might reflect increased interoceptive awareness in alexithymia. We assume that increased awareness of bodily and stress responses in alexithymics results in unspecific emotional arousal. Alexithymics mainly use suppression as a strategy to down-regulate emotional arousal, which might be reflected in neuronal activity decreases as mediated by the here reported increased GABA concentration in ACC. These hypotheses should be tested in further studies, though, that combine neuroimaging during emotional processing and interoceptive awareness with MRS measurements in insula and ACC in healthy subjects as well as in patients with affective disorders.

2.4 Author contributions

Study 1

Jutta Ernst, Simone Grimm and Georg Northoff designed the study. Heinz Böker and Jutta Ernst were responsible for recruitment. Jutta Ernst was responsible for scanning and clinical assessment/testing. Jutta Ernst and Simone Grimm undertook the statistical analyses. All authors contributed to the discussion and interpretation of the results. Jutta Ernst wrote the manuscript. All authors contributed to and have approved the final manuscript.

Study 2

Jutta Ernst, Simone Grimm and Georg Northoff designed the study. Heinz Böker and Jutta Ernst were responsible for recruitment. Jutta Ernst was responsible scanning and clinical assessment/testing. Jutta Ernst and Simone Grimm undertook the statistical analyses. All authors contributed to the discussion and interpretation of the results. Jutta Ernst wrote the manuscript. All authors contributed to and have approved the final manuscript.

3. General discussion

3.1 Synopsis

The work presented in the previous two studies was conducted using different imaging techniques, to gather not only information about neural acticity but also about the biochemical underpinnings. The following objectives were set: The aim of the first study was to investigate how empathy related neural activity in the interoceptive network is modulated by preceding interoceptive awareness. We hypothesized that the neural activity during empathy in these regions is enhanced by preceding interoceptive awareness (when compared with empathy preceded by exteroceptive awareness or empathy without any preceding awareness). Our second aim was to investigate whether regions of the empathy network are differentially modulated during empathy after interoceptive compared with exteroceptive awareness. We hypothesized that the preceding interoceptive awareness would significantly enhance neural activity during empathy in regions of the empathy network. For the second study, we investigated the association of interoceptive awareness with alexithymic features. The aim was to examine the association between alexithymic features, interoceptive awareness and glutamate and GABA concentrations in the left insula and ACC of healthy subjects. We hypothesized a relationship between interoceptive awareness and alexithymic features. For the insula, we predicted an association between increased interoceptive awareness, alexithymic features and glutamate concentration.

Recent studies suggested a significant relationship between interoceptive awareness and subjective emotional experience (Wiens and Plamer, 2001, Werner et al., 2009). Empathy, the experience of an affective or sensory state similar to that shown by a perceived individual, is closely related to emotional experience. While the affective component of empathy may therefore implicate interoception and interoceptive awareness, the impact of interoception on empathy has never been evaluated. In study 1 we tested how a preceding period of

interoceptive awareness impacts and modulates neural activity during subsequent empathy and found that the preceding interoceptive awareness period significantly enhanced neural activity during empathy in bilateral anterior insula and anterior cingulate cortex (ACC).

Alexithymia is a personality trait not only characterized by difficulties in identifying and describing feelings, but also by distinguishing feelings from bodily sensations of emotional arousal, which suggests a close association with altered interoceptive awareness. Despite the specific role of both insula and ACC in alexithymia as well as in interoceptive awareness and the behavioral relevance of altered neurotransmission in these regions, the respective relationships have not been elucidated yet. In study 2 we therefore first examined the relationship between alexithymic facets and interoceptive awareness and second, we investigated their association with glutamate and gamma-aminobutyric acid (GABA) concentrations in the left insula and the ACC using 3-Tesla proton magnetic resonance spectroscopy. Our results show a close association between alexithymia and interoceptive awareness. Furthermore, we found that glutamate levels in the left insula were positively associated with both alexithymia and awareness of autonomic nervous system reactivity, while GABA concentrations in ACC were selectively associated with alexithymia.

Combined, results from both studies demonstrate close association of interoceptive awareness with empathy and alexithymia. The enhancement of neural activity during empathy in both interoceptive and empathy networks by preceding interoceptive awareness suggests that interoception is implicated in empathy. Furthermore, our data might indicate that increased glutamate-mediated excitatory transmission—related to enhanced insula activity—reflects increased interoceptive awareness in alexithymia. Suppression of the unspecific emotional arousal evoked by increased awareness of bodily responses in alexithymics might thus be reflected in decreased neuronal activity mediated by increased GABA concentration in ACC.

Our first study investigated the relationship between interoception and empathy. Our results

showed that preceding interoceptive awareness induces enhancement of neural activity during empathy in both interoceptive and empathy networks. The bilateral insula has been shown to be important in both interoception (Critchley et al., 2004, 2005; Wiebking et al., 2010 ; Craig, 2002, 2009, 2010 ; Paulus et al., 2007) and empathy (Fan et al., 2011). Naqvi and Bechara (2010) have proposed that, through its connections with the ventral striatum, the insula acts as a "gate" through which memories for the interoceptive effects of experiences can motivate future experience-seeking behaviors. Indeed, insula activation is seen not only with the elicitation of primary emotions (Phan et al., 2002), but also with appetitive feeling states (Craig, 2010) and during financial decision making (Knutson et al., 2007). We showed that preceding interoceptive awareness enhanced neural activity during subsequent empathy when compared with either empathy with exteroceptive awareness or no preceding awareness period at all. The ability to share the feelings of others (and their internal states more generally) is thought to depend on premotor and sensory systems, including those for the perception of physical pain (Decety and Batson, 2007; Gallese et al., 2004). Limbic regions with the insula as a relay station and mirror neurons are activated by the observation of emotional facial expression (Watt, 2007). Plenty of studies have investigated how empathy and its underlying neural activity depend on exteroceptive stimuli and the exteroceptive context (Fan et al., 2010; Singer and Lamm, 2009; Decety et al., 2006; Lamm et al., 2007, 2010, 2011; Schnell et al., 2011), but no study demonstrated how the variation of interoceptive stimuli impacts empathy. One would hypothesize that variation of the interoceptive state of one's body also impacts the degree of neural activity and possibly the behavioral manifestation of empathy itself.

In our second study we indicated for the first time a close relationship between alexithymia, interoceptive awareness and GABA and Glu concentrations in ACC and insula. IA and alexithymia seem to share inversely associated abilities or functions that are involved in the perception, processing, and integration of internal bodily signals and emotions into emotional

experience and self-awareness. Both alexithymia and the feeling of the body state, which constitute a representation of the "material me" or the "physiological condition of the body" (Craig, 2002), are mediated by overlapping neural structures and pathways, such as the ACC and the insula and its frontal connections (Borsci et al., 2009; Craig, 2009; Critchley et al., 2004; Lane, 2000) which is in line with our results. Lane et al. (1997) was one of the first to propose that a dysfunction of the ACC underlies the problems in the conscious experience of emotions observed in alexithymia. A recent meta-analysis showed increased activation in the dorsal ACC but various studies on alexithymia reported decreased ACC activation during emotion processing. Pouga et al. (2010) and Kano and Fukudo (2013) suggested that these discrepancies regarding ACC activation might be due to differences in task paradigms and stimuli between studies. Notably, most studies reporting lower activation in the dorsal ACC during emotion processing in association with alexithymia (Kano et al., 2003, Karlsson et al., 2008 and Moriguchi et al., 2007) applied tasks, which require more cognitive processing of the emotional stimuli. GABA has a key role in cognitive processing. Yoon et al. (2010) showed correlations between GABA levels and task performance, using a contrast discrimination task. By showing a correlation with TAS scores and GABA in the ACC we can support that GABA has a key role in cognitive processing. Besides playing an important role in alexithymia the ACC is also a crucial region for emotion processing and for constituting our sense of self (McKiernan et al., 2006; Northoff et al., 2006). It has been hypothesized that while insula is involved in the generation of all subjective feeling states, combined action of insula and ACC might provide the neural basis of self-awareness (Craig, 2009). Medford and Critchley (2010) proposed that awareness of self, i.e. an integrated awareness of cognitive, affective and physical state is generated by the integrative functions of the insula and then re-represented in ACC as a basis for responses to inner or outer events. Back-projections from the ACC may then allow the insular representation of the feeling state to be modulated by cingulate activity. The proposed reciprocity between ACC and insula is supported by neuroanatomical studies (Nieuwenhuys et al., 2008; Moisset et al., 2010), findings of correlated BOLD signal

fluctuations (Taylor et al., 2009; Horn et al., 2010) and joint activity of these areas during emotional experience (Harrison et al., 2008). Menon and Uddin (2010) characterize insula and ACC as a 'salience network' that functions to segregate the most relevant among internal and external stimuli in order to guide behavior. The insula regulates not only the autonomic activity in in reaction to salent stimuli but is also directly involved in pain perception (Craig et al., 2000; Ostrowsky et al., 2002). Furthermore, the insula takes part in the cognitive processing of emotions as well as in the generation of emotional states (Medford and Critchley, 2010; Phillips et al., 2003), and is a key region in the subjective experience of feelings derived from bodily states and emotional arousal (Critchley et al., 2004; Diekhof et al., 2011). Recently, insula activity was found to be reduced in an empathy-for-pain experiment in high alexithymics compared to low alexithymics (Bird et al., 2010). Similarly, Silani et al. (2008) reported that reduced anterior insula activity is associated with less emotional awareness in interoception. From these findings, it can be concluded that alexithymic traits might be linked to difficulties to engage (anterior) insula when focusing on emotions and a failure to simulate forward representations of bodily states within the insula (Silani et al., 2008; Singer et al., 2009). Recent studies showed altered Glu levels in insula in acute pain and chronic pain and have been further discussed as an indicator for amplified interoceptive sensory processing (Harris et al., 2008; Gussew et al., 2010). Harm avoidance and extraversion have been associated with increased and decreased ACC GABA concentrations (Kim et al., 2009; Goto et al., 2010). That pain is also important in alexithymia showed a study where significantly higher pain scores were found in the alexithymic group compared to the non-alexithymic group (Saariaho et al., 2013). Our results support this hypothesis and we were able the show for the first time the relationship between alexythmyia and neurotransmitter concentrations. Alexithymics show increased vulnerability to affective disorders, where studies have reported increased interoceptive awareness and insula activity, which might reflect the patients' inability to shift the focus of perception/awareness from the own body to the environment (Grimm et al., 2009; Wiebking et al., 2010). Likewise, the

positive association reported between insula Glu concentration and alexithymia might reflect enhanced insula activity in alexithymia related to increased Glu-mediated excitatory transmission.

3.2 Limitations of the present studies

Some methodological limitations of the studies need to be mentioned. First, our sample size was rather small. This needs to be taken into account when interpreting the results.

Interoception may have a general facilitative relationship to all forms of affect and affective experience but not a specific relationship to empathy per se, so future studies should investigate the impact of interoceptive awareness on other forms of emotional processing to answer this critical question. Second, although we investigated two regions related to alexithymia and interoceptive awareness, future studies should include a further control region. Since specifically the right insula might support interoceptive awareness and integrate it with other information to form the basis of the subjective experience of an emotional state (Craig, 2003), future studies may want to include the right insula as well. And finally, fMRI has unequalled spatial resolution. However the temporal resolution of FMRI is inherently limited by the slow blood flow response it depends. FMRI cannot uncover the dynamics of mental activity on the sub-millisecond timescale on which neurons operate.

One of the limitations of MRS is that it provides the metabolic composition of a given voxel, which may include more than one type of tissue. For some applications, the voxels are relatively large (e.g., greater than 1 cm^3), but by using a 3T (Tesla) MRI machine- like in our Study 2- they may be somewhat smaller. The 3T technique creates greater inhomogeneities, however, which require better shimming techniques (Sood et al., 2010). Another difficulty is the fact that millimolar concentrations of metabolites have to be studied in the presence of the dominating resonances of water and lipids. In addition, the chemical shift range for proton metabolites is very small, and as all metabolites contain protons, the spectrum is very

crowded. Finally, the line broadening due to static field nonhomogeneities is proportional to the nuclear gyromagnetic ratio, and the demands for shimming to achieve proper line shapes in a proton spectrum are much more severe than for the other nuclei. Thus, proton MRS methodological development has focused on minimizing unwanted resonances using water and lipid suppression techniques in addition to spectral editing. Fortunately, lipid suppression is straightforward in the brain, since the lipid signals originate from the tissue overlying the brain and are not present inside the brain. The development of spatial localization techniques has benefited brain MRS, by allowing any region inside the brain to be studied individually, without lipid contamination (Tran et al., 2000.).

3.3 Concluding remarks

Our investigations in healthy subjects with personality traits that increase depression risk underscore the role of glutamatergic neurotransmission for emotional processing. The consecutive fMRI/MRS in vivo approaches applied in our studies enabled us to integrate personality dimensions into a functional/metabolic framework and to reveal how cellular mechanisms might modulate brain function. Therefore, a change from a descriptive to a causal level of in vivo was attained. Our findings may guide future investigations on novel therapeutic targets in depressive subjects. Based on the depicted link between personality traits and altered brain metabolism and function, the latter might be used as supporting objective measures and possible biomarkers for increased depression risk. Furthermore, individuals with alexithymia report lower life satisfaction and are more likely to commit suicide, so it is of great clinical importance to gain more insight in the neural basis underlying this personality trait.

Previous studies of neural/biochemical changes in insula and ACC have focused on empathy and alexithymia alone, not their association with interoceptive awareness.

By showing the direct relationship between empathy and interoceptive awareness in our first

study we demonstrated the important role of interoceptive components in empathy and that these components may need to be added to the other various components, such as sensorimotor, affective, and cognitive functions contributing to empathy (Schnell et al., 2011). This bears important clinical implications, especially with regard to affective disorders since patients with depression often show impaired empathic abilities like significantly lower levels of both cognitive (Perspective Taking) and affective (Empathic Concern) empathy (Cusi et al., 2011). Our findings confirm previous reports of a quite consistent empathy network that comprises the bilateral anterior insula, the ACC, the thalamus, and the medial prefrontal cortex (Fan et al., 2010, 2011; Molenberghs et al., 2011; Singer and Lamm, 2009; Decety et al., 2006; Lamm et al., 2007, 2011). However, we extend these results by showing an enhancement of neural activity during empathy in both interoceptive and empathy networks by preceding interoceptive awareness, which suggests a close relationship between interoception and empathy.

Despite the specific role of both insula and ACC in alexithymia as well as in interoceptive awareness and the behavioral relevance of altered neurotransmission in these regions, the respective relationships have not been elucidated yet. We showed that increased Glu-mediated excitatory transmission and related enhanced insula activity might reflect increased interoceptive awareness in alexithymia. We further report an assocation between GABA and alexithymia. Previous studies showed that alexithymics mainly use suppression as a strategy to down-regulate emotional arousal (Helmes et al., 2008), which is also reflected by lower ACC activation in response to emotion stimuli (Leweke et al., 2004; Moriguchi et al., 2007; Karlsson et al., 2008; Silani et al., 2008; Bird et al., 2010; Reker et al., 2010). These neuronal activity decreases might be mediated by the here reported increased GABA concentration in ACC. To sum up, our findings support the important role of glutamate and GABA in alexithymia. Given that both dysfunctional neurotransmission and alexithymia are associated with affective disorders, these results bear important clinical implications and might allow us to identify either subjects at risk or specific subgroups of depressive patients. Since it is still

unknown which factors are crucial for emergence of depressive symptoms, it is of special importance to better for factors that might increase depression risk and to characterize their neurobiological underpinnings. Finally, the results may also lead to a better understanding of the altered neurotransmission in depression.

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Reviewer activity

Neuroscience Letters
Journal of Visualized Experiments
European Journal of Pain

Talks and Poster Presentation

• DGPPN 2009, 2010, 2011, 2012, 2013

• SOBP 2013

• WFSBP 2009